

**JOINT MANIFESTATIONS IN INFLAMMATORY BOWEL
DISEASE (IBD) – A CROSS-SECTIONAL STUDY WITH
SPECIAL EMPHASIS ON RELATIONSHIP OF GUT
FLORA TO BOWEL AND JOINT DISEASE**

A dissertation submitted to the
Tamil Nadu Dr. MGR Medical University,
Chennai

Doctor of Medicine (D.M)
Branch IV
(Gastroenterology)

August 2010

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Guided by
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Submitted by
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August 2010

<u>DECLARATION</u>

I, Dr. B. Avinash hereby declare that this dissertation entitled “Joint manifestations in Inflammatory Bowel Disease (IBD) – A cross-sectional study with special emphasis on relationship of gut flora to bowel and joint disease” has been prepared by me under the direct supervision and guidance of Dr. B.S Ramakrishna, DM, Professor and Head of Clinical Gastroenterology & Hepatology, Christian Medical College, Vellore being submitted to Dr M.G.R medical university in partial fulfillment of regulations for the award of DM degree in gastrointestinal sciences examination to be held in 2010.

This dissertation has not been submitted by me either in part or in full on any previous occasion to any university or institution for the award of any degree or diploma.

Place: Vellore

Date:

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**DEPARTMENT OF GASTROINTESTINAL SCIENCES
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CERTIFICATE

This is to certify that the dissertation entitled “Joint manifestations in Inflammatory Bowel Disease (IBD) – A cross-sectional study with special emphasis on relationship of gut flora to bowel and joint disease” is a bonafide work done by Dr. B. Avinash under my guidance and supervision, in partial fulfillment of the requirements laid down by the Tamilnadu Dr. M.G.R Medical University, for the award of DM degree in Gastroenterology, examination to be held in 2010.

Place: Vellore

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**Christian Medical College,
Vellore**

Dr. Avinash

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INTRODUCTION

Joint involvement is the most common extra intestinal involvement in inflammatory bowel disease (IBD) and occurs in 10-20 % of the patients. Peripheral involvement is pauciarticular, asymmetrical, non-deforming, transitory and migratory synovitis and or arthritis. Peripheral enthesiopathies can occur at Achilles tendon and at the insertion of fascia plantaris. Axial involvement can vary from asymptomatic sacroiliitis to classic ankylosing spondylitis .The true prevalence of axial involvement is not known as the onset is insidious. There is parallelism between the flare up/remission of bowel symptoms and arthritis suggesting a similar etiopathogenesis. Inflammatory peripheral arthropathy is similar to the reactive arthritis that occur secondary to certain intestinal infections. Reactive arthritis is secondary to a small set of intracellular, aerobic, invasive gram negative bacteria and is dependent on specific properties of the bacterium. The temporal profile of the gut and joint inflammation suggests the involvement of the bowel bacterial flora. However, the definite profile of the flora responsible for this is not known. Chronically disabled persons can disorganize the life of the entire family and disrupt the existing balance between them. Joint manifestations leading to disability impairs the patient's quality of life and is a burden to the family.

IBD is an inflammatory condition of the bowel; a clinically heterogenous disorder with the potential for systemic involvement. A wide clinical spectrum may be observed including tendinitis, enthesopathy, peripheral arthritis, axial arthritis (sacroiliitis) or a

combination of these complications. Patients with IBD presenting with inflammatory back pain and /or peripheral arthritis are classified as spondyloarthropathy. Inflammation of the colon (ulcerative colitis or Crohn's colitis) leads to joint manifestations in 26-39% of patients, whereas patients with Crohn's ileitis develop joint manifestations in only 14%; this reflects the importance of the colonic bacteria in the causation of arthritis. Inflammatory bowel disease is believed to result from an abnormal immune reaction to luminal bacteria in a genetically predisposed host. In addition to host immune and genetic factors, the luminal bacteria are also considered to be important. Bacteria in the intestine probably prime immune cells that then circulate to the joints and damage them. While several investigators have described differences in the intestinal microbial flora between patients with IBD and healthy individuals, it is not known whether IBD patients with arthritis have a different flora from IBD patients without arthritis. We know little about the pattern of joint involvement in IBD in India and how disabling it is to the person who suffers from IBD. It would be interesting to know this because the genetic background of Indians is probably different from Caucasians where the data is available and we know that the course of disease in Indians is probably different, eg., less commonly leading to cancer. We therefore proposed to study the prevalence of joint disease in patients with IBD, to document its pattern, and to determine whether the gut bacteria of patients with joint involvement are different from those of patients without joint involvement.

<u>ABSTRACT</u>

TITLE OF THE ABSTRACT: Joint manifestations in Inflammatory Bowel Disease (IBD) – A cross-sectional study with special emphasis on relationship of gut flora to bowel and joint disease

DEPARTMENT: Gastrointestinal Sciences

NAME OF THE CANDIDATE: Dr Avinash B

DEGREE AND SUBJECT: Doctor of Medicine (D.M) Medical Gastroenterology

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OBJECTIVES : To assess the frequency and pattern of joint involvement in inflammatory bowel disease and to determine whether joint involvement is associated with a dysbiosis of the intestinal microbial flora

METHODS: Consecutive patients with ulcerative colitis (UC) or Crohn's disease (CD) were prospectively interviewed and examined by the principal investigator and clinical details and laboratory results noted. The pattern of joint involvement and severity was independently confirmed by a specialist rheumatologist. Radiographs of the involved peripheral joints, sacroiliac joints and spine were obtained. DNA was extracted from stool samples and real time PCR targeting 16S ribosomal DNA was used to quantitate specific classes of “beneficial” (*Clostridium coccoides*, bifidobacteria, and lactobacilli) and “aggressive” (*Bacteroides*, Enterobacteriaceae, Enterococcus) bacterial flora. Disease

activity and bacterial counts were compared between patients with and without joint involvement.

RESULTS : Sixty eight patients with ulcerative colitis (UC) and 60 with Crohn's disease (CD) were included in this prospective study. Arthritis was noted in twenty- four IBD patients (18 %); 13 with CD (21.6%) and in 11 with UC (16.1%). Peripheral arthritis alone was noted in 12 patients, axial involvement alone in 7 and rest had mixed pattern. The most prevalent phyla in IBD patients were Bacteroidetes and Bifdobacterium.. Enterobacteriaceae were significantly higher in patients with UC than in CD. In UC patients, joint involvement was associated with higher Enterococcus species and lower Clostridium coccoides suggesting a dysbiosis in these patients.

OBJECTIVES AND AIMS OF STUDY

The overall aim of this study was to assess the frequency and pattern of joint involvement in Indian patients with inflammatory bowel disease and to determine whether specific classes of the intestinal microbial flora of IBD patients with arthritis was quantitatively different from that of IBD patients without arthritis. The specific objectives were as follows:

- To study the prevalence, pattern and disability resulting from joint disease in patients of inflammatory bowel disease in India.
- To study the pattern of bacterial flora of the gut in patients with IBD and arthritis and to compare this with the pattern in patients with IBD but without arthritis.

REVIEW OF LITERATURE

History:

The first author to report the association between arthritis and Ulcerative Colitis (UC) was White in 1895 while the first report of joint disease in Crohn's Disease (CD) may be described by Abercrombie in 1930. Both were post mortem examination findings in patients of arthritic symptoms (*Jan tore gran et al*). The association between disease of bowel and musculoskeletal symptoms was recognized since 1900. In 1920, Rea Smith described bowel infection was responsible for rheumatism and to treat rheumatic complaints he performed partial colectomy in 68 patients. Hench described peripheral joint arthritis associated with chronic ulcerative colitis in 1935. He had noted flares of arthritic symptoms with colitic flares. In 1959, Wigut and Watkinson described arthritis associated with inflammatory bowel disease (IBD) (*Gravallese et al*). It was believed that colonic stasis be it anatomic or functional in origin fostered bacterial fermentation which produced arthritis. The current opinion favors the concept of enteric organisms acting as bowel triggers in disease susceptible individuals (*Jan tore gran et al*)

IBD is divided into two major clinical pathologic subtypes – Ulcerative Colitis (UC) and Crohns Disease (CD). The articular complications of each are considered identical. Joint disease is the most common extraintestinal features of IBD. A wide spectrum of joint manifestations have been observed in IBD including tendonitis, enthesopathy, peripheral (non deforming) arthritis, axial arthritis (sacroilitis) or a combination of these complications. (*Peter et al*).

Epidemiology:

Prevalence of UC ranges from 50 – 100 per 100000 in the general population. Prevalence of CD has been increasing during the last few decades to about 75 per 100000. In a screening study for colorectal cancer involving 37000 individuals without intestinal symptoms the combined prevalence of UC and CD was 56 per 100000 whereas the prevalence of symptomatic IBD is estimated as 90-150 per 100000 (*Mayberry et al*) . The reported incidence of enteropathic arthritis in IBD varied among authors. In the original monograph on Crohn's disease only brief mention of arthritis is made as a complication. Earlier studies suggested that arthritis was noted commonly in UC than in CD but more recent studies suggest the opposite. Farmer et al studied a cohort of patients with CD strictly defined into small intestinal, ileocolonic and colonic involvement at the time of initial presentation. Peripheral arthritis was seen in 16% of patients with colonic involvement compared with 4% of those with small intestinal involvement and 3.6% with ileocolonic involvement. Of patients with CD, those with colonic involvement alone had the highest incidence of peripheral arthritis. The axial arthropathy in CD is identical to that in UC in frequency of occurrence and in clinical manifestations. The reported incidence of spondylitis in association with IBD varies from 1.1% to 6.4%. Dekker Saeys et al studied 109 consecutive patients with IBD along with extensive rheumatologic examination of all patients and noted that spondylitis was present in 3.7% of the cases. Arthritis is the most common extra intestinal manifestation of IBD and appears in 2-20% of patients with either UC or CD. The frequency of peripheral arthritis in IBD ranges from 17-20% of patients with a higher prevalence in CD. (*Gravallese et al*)

In an extensive retrospective study in an Oxford IBD clinic involving 1459 patients with IBD, peripheral arthritis was described in 6% of UC and 10% of CD.

(Orchard *et al*) In a study from gastrointestinal clinic, actual synovitis was noted in 10% of IBD patients, enthesitis in 7% and 29% of patients reported a history of swollen joints. Axial involvement occurs in both the diseases. The true prevalence of sacroilitis in IBD is unclear as the onset is usually insidious. Thirty percent of patients with IBD had inflammatory back pain, 1/3rd of patients had unilateral or bilateral sacroilitis (SI) stage II and in 18% of patients SI was asymptomatic. Kochhar *et al* in a prospective study of 150 IBD patients noted radiological sacroilitis in 14%, peripheral arthritis in 10.7% without a single case of spondylitis. This was in contrast with other Indian studies that had reported fewer incidences of extraintestinal features in the Indian population. (R Kochhar *et al*). Polyarticular destructive arthritis and unilateral sacroilitis may also be manifestations of CD (Norton *et al*)

The spondyloarthropathies and IBD likely involve a poorly understood interaction of genetic and environmental factors. Common features include a genetic predisposition, a triggering antigen, and an abnormal immune response (Joseph McKinley *et al*)

Evidence of Gut-Joint Enteropathy in peripheral arthritis

Inflammatory peripheral arthropathy in IBD is similar to reactive arthritis secondary to intestinal infection. In the latter, aerobic invasive Gram negative bacteria e.g. *Shigella flexneri*, *Salmonella typhimurium*, *Yersinia enterocolitica* and *Campylobacter jejuni* are the possible etiological agents. However other organisms with similar invasive properties such as enteroinvasive *Escherichia coli* have shown no relation with reactive arthritis. These observations indicate that the specific properties of the bacterium are important. The clinical observation of parallelism between the flare up of CD and peripheral arthritis

suggests a similar etiopathogenesis. There is no data on the presence of bacterial DNA /RNA/ degradation products in the synovium. The best evidence of bacterial role of the arthritis in IBD comes from transgenic rats in which colitis and arthritis develop only after restoration of gut flora. (*De Vos et al*)

Exacerbations of bowel disease and flares of peripheral arthritis tend to coincide in both UC and CD. Greenstein et al showed that the incidence of peripheral arthritis tends to be higher in patients with CD than in UC. Of patients with CD, those with colonic involvement alone had the highest incidence of peripheral arthritis. In patients of UC, total colectomy may prevent further attacks of peripheral arthritis and such surgery does not typically prevent recurrent arthritis in CD. The possible pathogenesis being that the diseased bowel may provide the immunological stimulus for the occurrence of arthritis. In the case of UC, total colectomy provides removal of all diseased bowel and thereby removal of arthritogenic stimulus. In CD surgical removal of the entire diseased bowel is unusual. (*Gravallese et al*)

The peripheral arthropathy associated with either UC or CD involving the colon is more commonly observed during an exacerbation of bowel symptoms, is common in patients with chronic inflammatory bowel disease than in individuals with an acute fulminant colitis and is more likely seen in patients with universal or left sided colitis than in those with inflammation limited to rectosigmoid area. The incidence of either the peripheral arthropathy or ankylosing spondylitis is not increased by the presence of intestinal complications such as fistula or stricture with either UC or CD. (*Thomas Danzi et al*)

Evidence of Gut-Joint Enteropathy in axial involvement

Two forms of axial involvement are noted in IBD. The first is spondylitis which includes sacroiliitis and is indistinguishable clinically and radiologically from idiopathic ankylosing spondylitis. The second is an isolated sacroiliitis which is often asymptomatic. (*Gravallese et al*). The degree of peripheral joint involvement appears to correlate with the degree of bowel inflammation whereas the spondyloarthropathies tend to progress without relation to the intestinal disease. (Norton et al)

Axial involvement in IBD can vary from asymptomatic sacroiliitis to classic ankylosing spondylitis. Radiographic involvement of sacroiliitis of at least grade II was noted in 11% of CD patients. The prevalence varies from 3% to 10%. There are some observations which suggest alterations in gut flora especially the presence of *Klebsiella* in the gut but the presence of bacterial agents in axial joints has generally not been detected low due to low joint accessibility. The contrary is also true with the presence of gut alterations in about 60% of spondyloarthropathy patients varying from increased lymphoid follicles to chronic intestinal inflammation. (*De Vos et al*)

Although there is no increased incidence of HLA B27 in enteropathic peripheral arthritis, there is reportedly an increased incidence of HLA B27, 53% - 75% in patients with spondylitis and IBD. Relatives of patients with IBD have higher spinal inflammation though the probands have no evidence of spinal involvement suggesting a genetic predisposition. (*Gravallese et al*)

De Vos et al demonstrated a gradient increase in the prevalence of HLA B27 from 0% in CD with inflammatory back pain only to over 14% in patients with isolated

sacroiliitis and upto 78% in patients with ankylosing spondylitis suggesting that evolution to ankylosing spondylitis is more likely in HLA B27 positive patients. (*De Vos et al*)

Proposed etiopathogenesis mechanisms in gut joint involvement

A number of bacterial agents such as Gram negative invasive *E. coli* and Gram negative anaerobic rods of *Bacteroides* and *Fusobacterium* are implicated in the etiopathogenesis of CD. Similar bacterial agents are involved in the etiopathogenesis of spondyloarthropathy.

Intestinal bacteria may reach the dendritic cells and macrophages in different ways - transport by M cells; uptake and processing by epithelial cells; direct access to dendrites located between epithelial cells and direct access to antigen presenting cells through the breaks in the epithelial barrier.

Polymorphisms of CARD 15 are associated with CD and also it has role in bacterial handling. CARD 15 is located on the chromosome 16 and encodes for an intracellular protein with binding affinity to bacterial muramyl peptide. CARD 15 protein is expressed by monocytes, macrophages and dendritic cells. *In vitro* it induces the activation of nuclear factor κ B pathway after recognition of muramyl peptide. It helps in protecting the gut wall against aggression. Genetic modulation of CARD 15 expression may lead to disturbed handling of bacterial products/inappropriate elimination.

T cells in the lamina propria of CD and spondyloarthropathy patients produce interferon γ resulting in production of proinflammatory cytokines (tumour necrosis factor α , interleukin 6 and interleukin 1). Effector T cells migrate from inductive sites to adhesive sites and the expression of the adhesion molecules regulate the cell traffic.

Intestinally activated T cells may enter the synovium either by the presence of cognate antigens at both sites or by homing of lymphocytes primed in gut by $\alpha 4\beta 7$ and vascular adhesion protein 1 (VAP1). The discovery of identical T cell expansions in colonic mucosa, synovium and blood support this concept of gut – joint iteropathy. (*Ekkehard et al*)

There are numerous case reports in the literature describing IBD with persistent erosive monoarthritis. Pathological examination of the synovial tissue revealed chronic synovitis with single or multiple noncaseating epitheloid granulomas. The granulomas were similar to those noted in involved bowel wall. (*Gravallese et al*)

Enteropathic arthritis

The clinical characteristics of arthritic flares are similar in both UC and CD. Palumbo *et al* reviewed the articular manifestations of 121 patients with IBD and found no distinguishing characteristics between the arthritis that is seen in CD and that seen in UC. Joints most commonly involved in arthritic flares are knees, ankles, elbows and wrists followed by proximal interphalangeal joints, metacarpophalangeal joints and metatarsophalangeal joints. Knees and ankles are the joints that are most commonly involved in first attacks. Hips and shoulders are less commonly involved in patients without associated spondylitis. The onset of arthritis may be insidious. Over 90% of attacks are polyarticular and a migratory pattern of joint involvement is noted in 50% of the patients. In fifty percent of the patients the duration of arthritic symptoms lasted less than 1 month; 25% of attacks lasted 5 weeks to 2 months; 10% of attacks lasted 2-6 months and 10% lasted 6-12 months. In the remaining 5% of the cases a chronic arthritis

lasting for several years ensued. The peripheral arthritis associated with IBD is of non-deforming and non destructive type. Although pain and limitation of joint motion and joint effusion are common clinical findings at flares these often resolve without sequelae. (*Gravallese et al*)

Orchard *et al* analysed the natural history and articular distribution of peripheral joint involvement without axial disease in a cohort of IBD patients. They described 2 forms of peripheral arthropathy. Type 1 (pauciarticular) characterised by self limiting attacks of oligoarthritis (less than 5 joints) that often coincided with relapses of IBD. Type 1 occurred in 4.4% of the patients and was associated with HLA B 27 in 26% of the patients. Type 2 (polyarticular) was noted to involve more than 5 joints, occurred in 2.9% of IBD patients, with a more severe course of symptoms persisting for months to years independent of the IBD activity. (*Orchard et al*) Salvarani *et al* in a population based study of 202 IBD patients did not observe any association between peripheral arthritis of IBD and HLA B 27 antigen. (Salvarani *et al*)

The clinical features of spondylitis associated with IBD are similar to those seen in idiopathic ankylosing spondylitis. The low back pain is insidious in onset with morning stiffness. Pain is usually better after exercise and worse with rest. Physical examination reveals limited spinal flexion and reduced chest expansion. Spondylitis in IBD can occur at any age. In contrast to the peripheral arthritis associated with IBD, symptoms of spondylitis tend to be unrelated to the exacerbations and remissions of bowel disease. Axial symptoms usually occur after onset of bowel symptoms but may precede the bowel symptoms. Distinct from the enteropathic peripheral arthritis is an arthritis involving large joints seen specifically in patients with spondylitis. The hips and

shoulders are specifically involved and to a lesser extent the knees. Hips and shoulders may be permanently damaged. (*Gravallese et al*)

Classification of enteropathic peripheral arthropathy (Orchard et al)

Type 1 (pauciarticular)

- + Less than 5 joints
- + Acute, self-limiting attacks (<10 weeks)
- + Often coincides with relapses of IBD
- + Strongly associated with extraintestinal manifestations of IBD

Type 2 (polyarticular)

- + Five or more joints
- + Symptoms usually persist for months to years
- + Runs a course independent of IBD
- + Associated with uveitis but not with other extraintestinal manifestations

Relevant gut biology:

The adult human gastrointestinal tract is not a complete barrier, being permeable to some macromolecules. Permeability is increased in certain disease states, including inflammatory bowel disease, celiac disease, and the administration of nonsteroidal antiinflammatory drugs. Thus, the adult gastrointestinal tract is a potential source for exposure to both bacterial and dietary antigens, particularly when permeability is increased (*Bjarnason, et al., Smth et al.,*).

The human gastrointestinal tract plays host to a large number of bacteria, consisting of more than 400 species, their total number exceeding the number of cells in the human body. The large majority of these bacteria are anaerobic. These bacteria play an important role in human health by producing nutrients, preventing colonization of the gut by potential pathogens, and affecting immune responses (*Guarner et al; Ramakrishna et al*) The healthy gut harbors a mixture of native bacteria acquired at birth or shortly thereafter that retains a relatively constant composition; it also has a smaller population of transient bacteria of varying composition. The former are essential for health and live in symbiosis; the latter contain potential pathogens. Whereas the stomach and duodenum normally contain less than 10^3 mucosa-adhering bacteria, the number of bacteria increases to 10^4 in the jejunum and 10^7 in the ileum. Most of this last group is gram-negative aerobic species. In the colon, the bacterial density is 10^{12} or more, consisting mostly of anaerobic bacteria. Transit time is fast in the upper gut and slow in the distal gut, but the immunologic impact of the microflora is higher in the proximal parts of the gut. (*Guarner et al*)

Antigenic exposure via the gastrointestinal tract usually results in tolerance rather than immunity. Normal individuals appear to be tolerant to their own intestinal flora, but intolerant to that of others. Patients with active IBD lose tolerance to their own bacterial flora, an abnormality that is reversed when the disease comes under control. Whether this loss of tolerance is a cause or a consequence of the IBD is not known (*Duchmann et al.*) Enhanced mucosal permeability may play a pivotal role in maintaining a chronic inflammatory state, due to a genetic predisposition or as a result of direct contact with bacteria or their products . A defective epithelial barrier may cause a loss of tolerance to

the normal enteric flora. Furthermore, an increased mucosal absorption of viable bacteria and bacterial products is found in IBD. Serum and secreted antibodies are increased and mucosal T-lymphocytes that recognize luminal bacteria are present. However, there is evidence that the immune system reacts over aggressively towards the normal luminal flora rather than the flora being altered in IBD. Several approaches have been used in attempts to discover a specific microbial agent in the cause of IBD. These include demonstration of the presence of organisms or specific antigens in affected tissues, culture of microbes from the affected tissues, demonstration of serological responses to several agents, and localization and detection of individual pathogen-specific nucleic acid sequences in affected tissue by in situ hybridization and polymerase chain reaction. So far, no specific micro-organism has been directly associated with the pathogenesis of IBD. Analysis of the luminal enteric flora, however, has revealed differences in the composition of this flora compared to healthy controls . In Crohn disease, concentrations of *Bacteroides*, *Eubacteria* and *Peptostreptococcus* are increased, whereas *Bifidobacteria* numbers are significantly reduced. Furthermore, in ulcerative colitis, concentrations of facultative anaerobic bacteria are increased. (Linskens RK et al)

Gut associated lymphoid tissue

Gut-associated lymphoid tissue (GALT) is the largest lymphoid organ of the body, constituting 25% of the mucosal mass. The gastrointestinal tract has been called “the most misunderstood and underappreciated lymphoid organ of the body”. GALT components are found in Peyer's patches, gut lymphoid follicles, lamina propria, and intraepithelial T cells. From the Peyer's patches, primed B lymphocytes disseminate throughout the body's mucous membranes, notably to other parts of the alimentary tract.

Primed T lymphocytes also disseminate into the circulation and lymph nodes and home into target organs, such as salivary glands (in Sjögren's disease), lungs, and synovium. A chain of events in the pathogenesis of enteropathic arthritis can begin with gastrointestinal infection with the appropriate microorganism in a genetically predisposed patient. This causes local inflammation in the gut mucosa, formation of secretory IgA, increased permeability, absorption of foreign material, and triggering of T lymphocytes. Circulating immune complexes and memory T cells localize to joints and cause synovitis. (*Fumiko et al*)

The pathogenesis of ulcerative colitis (UC) remains unclear. The disease is characterized by exacerbations and remissions and runs a prolonged course. In addition to possible genetic factors concerned with immunoregulation and altered mucosal barrier function, dysbiosis which is an abnormal ratio of beneficial to detrimental commensal microbial agents has also been implicated in disease pathogenesis and relapse (*Balfour et al*) The normal resident intestinal flora prevents the overgrowth of pathogenic bacteria. *Clostridium difficile* is a Gram-positive anaerobic bacterium that is present as a very minor constituent of the colonic flora. The use of broad spectrum antibiotics disrupts the ecosystem of the normal colonic flora, and may predispose to colonization of harmful bacteria. (*Balamurugan et al*)

The bacterial answer to CD and ulcerative colitis (UC) could be revealed in the difference in the colonic flora between identical twins discordant for the disease. The twin data also suggest the organism needs to be present in 80% of people ($60 + 60 + 40 = 80\%$ of 200 individuals in 100 sets of identical twins). Antibodies to *Enterococcus faecalis* and *E. coli* have been found with increased frequency in affected bowel mucosa

of patients with CD. The ubiquitous presence of *E. coli* in the stool would not fit the discordant twin data; however, the 80% prevalence of enterococcus would. The immune handling of enterococcus is important in understanding the pathogenesis of IBD. (*Joseph McKinley et al*).

Joint manifestations of UC:

Frequency of peripheral arthritis in UC:

The reported prevalence of peripheral arthritis in UC varied from 0.9% to 34.6% in various studies. Out of 28 surveys, 19 studies concluded with a frequency of arthritis of less than 11%. However most of the studies were on hospitalized patients in whom there will be tendency of more association between the two disorders. Arthritis in UC was noted to be more prevalent in women than among men. In the majority of cases, colitis preceded the development of arthritis whereas in 0-25% of cases, the onset of arthritis antedated that of UC. A simultaneous occurrence of joint disease and UC was seen in no more than about 20% of cases. In a study by Mc Ewan et al., simultaneous flares of colitis and peripheral arthritis were observed in 60% of the patients. Arthritis tends to wax and wane with the activity of bowel disease and not every relapse of colitis will be followed by joint disease. In a survey by Wright and Watkinson, if rectum alone was affected only 4.9% had arthritis which sharply contrasted with the 14.4% incidence of arthritis among patients whose entire colon was affected. The joint distribution is most often asymmetric but symmetric is also common most frequently noted in the lower limbs. An attack of arthritis involves from 1-3 joints and less than 10% of cases have more than 6 joints involved. In 1952, Dennis and Karlson, showed that out of 19 patients

with UC and arthritis, all but 1 improved following removal of the colon and enjoyed complete loss of symptoms (*Jan tore gran et al*).

Frequency of Ankylosing Spondylitis (AS) and sacroiliitis in UC:

The first author to report the association between AS and UC was Romanus in 1953. Reported prevalence of AS in UC varies between 0.8 and 6.0%. Many studies concluded with the prevalence ranging between 3 and 6%. There is discrepancy regarding the date of onset of both the disorders. The clinical picture of AS develops without any consistent time or disease activity of UC and the relationship is based upon the disease susceptibility genes for one condition predisposing the other. Wright and Watkinson reported that sacroiliitis was observed in 13% of patients with rectal involvement as compared to 23.2% in whom the entire colon is affected. The radiological finding of sacroiliitis was noted to be a rather frequent feature of UC (18%) in contrast to AS which occurred in 3.3% of the cases. The prevalence of UC in AS was about 20 times higher than the general population. (*Jan tore gran et al*).

Joint manifestations of CD:

Frequency of peripheral arthritis in CD:

The prevalence of peripheral arthritis in CD varies from 0.4 to 23.0%. The peripheral arthritis of CD resembles that of UC. The joints most commonly involved are the knees and the ankles. Destruction of joints is rarely encountered. In the majority of patients the arthritis follows the bowel involvement. CD without colonic involvement has a reduced incidence of peripheral arthritis may support the notion that the distal part of the bowel plays a critical role in the development of joint manifestations. The beneficial effects of surgery upon arthritis are less pronounced in CD than in UC. According to other workers

the exacerbation of joint symptoms in CD subsequent to resection invariably heralds a recurrence of the intestinal lesion (*Jan tore gran et al*).

Frequency of Ankylosing Spondylitis (AS) and sacroiliitis in CD:

The prevalence of ankylosing spondylitis in CD varies between 2.0 % and 9.8% with most surveys estimating the prevalence between 2 and 4%. The clinical picture is similar to that in UC. The prevalence of CD in ankylosing spondylitis seems to be 40 – 50 times higher than that in general population (*Jan tore gran et al*).

Role of HLA B27:

The remarkably strong association of spondyloarthropathies (SpA) with the major histocompatibility complex (MHC) class I gene human leukocyte antigen (HLA)-B27 was first described in 1973. The HLA-B27 transgenic rat model represents a unique model to understand the role of the HLA-B27 gene in SpA with associated inflammatory bowel disease (IBD). The inflammatory cytokine pattern of HLAB27 transgenic rats (T helper [Th]1>Th2 cytokine expression) corresponds to that observed in Crohn's disease, while the histological features of their colitis closely resemble those of ulcerative colitis. Usually, IBD and arthritis symptoms do not develop under germ-free state but under pathogen conditions. In HLA-B27 transgenic rats, colitis is the earliest clinical manifestation, with diarrhea appearing after 10 weeks of age, while after several weeks of the onset of intestinal inflammation most affected rats develop peripheral arthritis. Recent evidence indicates that, in HLA-B27 transgenic rats, the presence of both CD4+ T cells and antigen-presenting cells (APCs) expressing high levels of HLA-B27 seems to be of critical importance in the pathogenesis of the disease. Because of the lack of a

demonstrable role of CD8⁺ T cells, recent hypotheses to explain the pathogenicity of HLA-B27 in this model are based on non-antigen-specific mechanisms, as opposed to the classic arthritogenic peptide-based hypotheses. This antigen-independent defect has been examined primarily in DCs and occurs at the level of contact between APCs and T cells. In these rats, APCs have relatively poor efficacy in stimulating T cells, and this could result in loss of tolerance toward self-antigens. Alternatively (or additionally), impaired T cell stimulation could result in altered control of gut bacteria, thereby sustaining stimulation of immune defense by macrophages. Therefore, IBD as well as arthritis symptoms can be prevented in “germ-free” conditions, while bacterial flora reconstitution fosters disease development (*Anna Farca et al*).

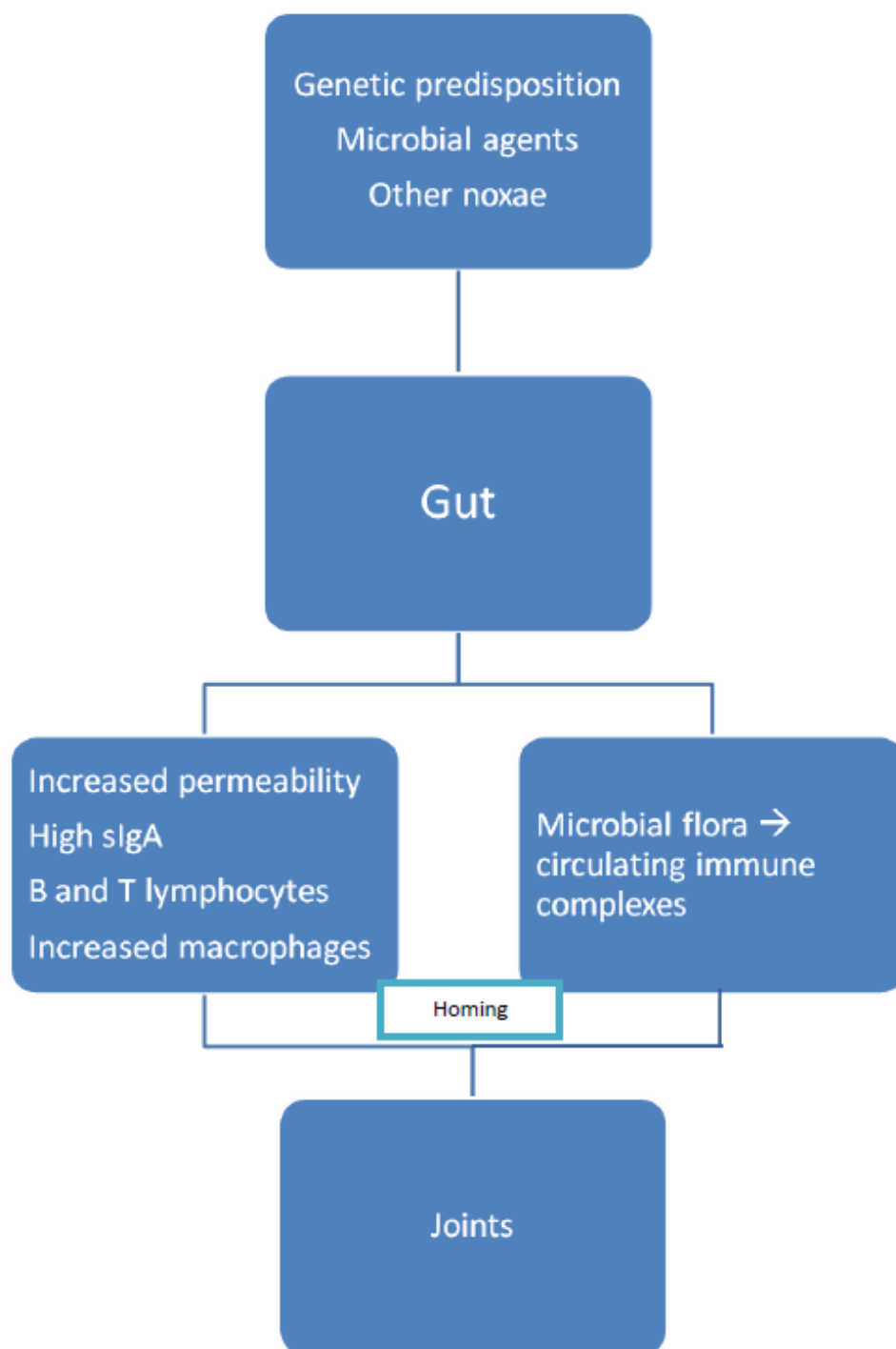


Fig. 1: Etiopathogenesis of Enteropathic arthritis

Treatment of enteropathic arthritis:

Therapy for rheumatological complications is secondary to treatment of the underlying IBD and generally directed towards reducing joint inflammation and preventing disability and deformity (especially axial symptoms). Guided physical therapy is of great importance in patients with axial arthropathy. Primary goals of physical therapy are to relieve pain, to secure optimal mobility and posture, to retard progressive clinical complaints and to reduce the consequences of spinal joint fusion. Sulphasalazine is the active moiety against both bowel and arthritic inflammation and should be considered a first line medical therapy. Non steroidal anti-inflammatory drugs (NSAIDS) are used frequently in arthritis but they may activate IBD. Intra articular steroids can have an effect that will last for months. Oral steroids are very effective. Azathioprine and methotrexate are appropriate for maintenance therapy. Recalcitrant disease may necessitate use of infliximab/ etanercept. (*Daniel et al*)

Summary of review of literature:

The diagnosis of enteropathic arthritis is made in a patient with IBD by the presence of asymmetrical, often migratory, non erosive and seronegative often migratory arthritis occurring most often in knee and ankle noted in 15- 20% of IBD more commonly in CD than in UC. As the arthritic flares accompany flares of underlying intestinal disease suggests a cause and effect relationship. An immunologic reaction to gut bacteria has been postulated in IBD. The therapy is usually directed at intestinal disorder and the peripheral arthritis tends to be corticosteroid responsive. It is not clear whether it is a true synovial response or steroids causing quiescence of bowel disease and thereby decreases in the immunologic stimulus for the ongoing arthritis.

Spondylitis occurs in 3-6% of patients with IBD with similar incidence in CD and UC. However radiological evidence of sacroilitis is reported more frequently (14-20%). The clinical course of axial arthropathy is unrelated to bowel disease activity and has an association with HLA B 27 which was distinct with that of the enteropathic peripheral arthritis. Cases in which spondylitis clearly precedes the onset of bowel disease argue against the cause and effect relationship.

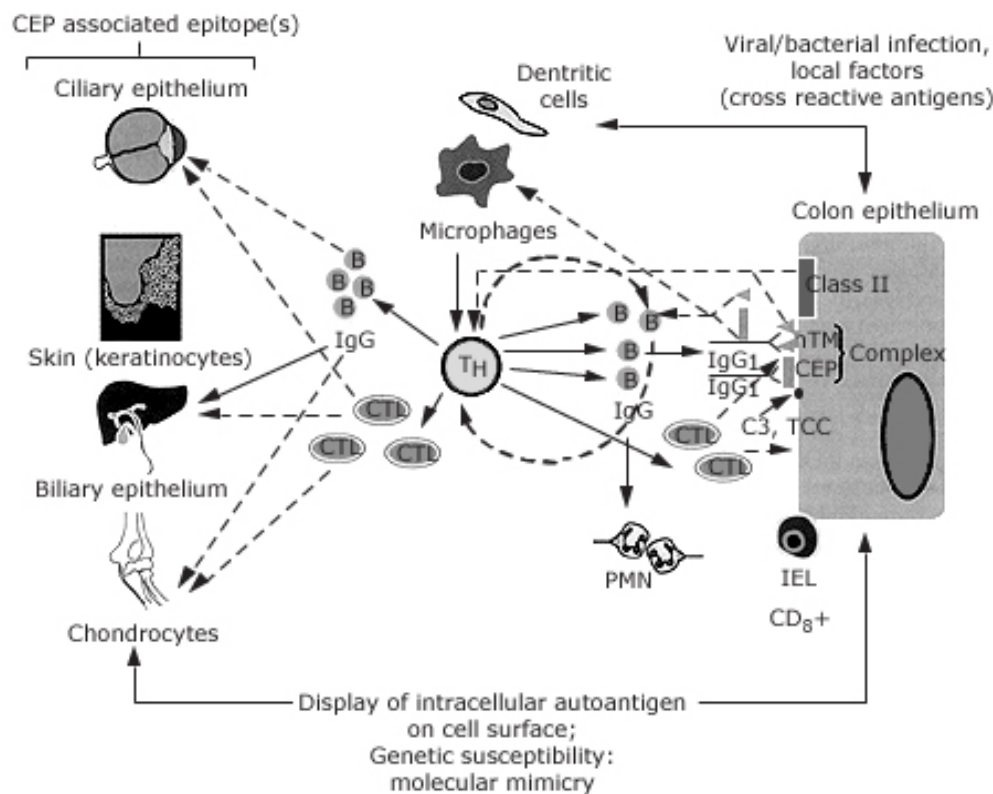


Fig 2: Proposed model of immunopathogenesis of ulcerative colitis and its extraintestinal manifestations

The model is based upon the unique distribution of a "shared autoantigen(s)" in the four extracolonic organs, namely eye, skin, biliary epithelium and joints. Because autoantibodies against human tropomyosin (hTM) isoform 5 and colon epithelial specific protein (CEP) have been demonstrated in UC and not in CD and other non-IBD controls, it is possible that both proteins, either individually or as an hTM+CEP complex, act as autoantigen(s) or are involved in the pathogenesis of UC. The autoantibodies belong to IgG1 subclass and can activate the complement system causing deposition of C3 and products of terminal complement complex (TCC) on the colonocytes in UC. Autoantigen-specific B cells can also act as APC, maintaining perpetuation of inflammatory process. Microbial peptide(s) crossreactive to the cellular autoantigenic epitope(s) (molecular mimicry), or release of cryptic peptide by local factors such as trauma, may initiate the autoimmune response. CEP/hTM5 associated epitope(s) are expressed at the selective extracolonic sites such as, non-pigmented ciliary epithelium in the eye, keratinocytes, biliary epithelium and chondrocytes. (*Das et al.*,)

PARTICIPANTS AND METHODS

A total of 128 consecutive patients with inflammatory bowel disease, seen between January 2007 and March 2009 at CMC, Vellore, were included in the study. Clinical information, laboratory and treatment data were collected for all patients by using uniform structured data forms (Proforma).

Participants:

Inclusion Criteria

All patients diagnosed to have IBD (ulcerative colitis or Crohn's disease) either newly or in the past and who were seen in the IBD or GE clinics.

Exclusion criteria

1. Failure to provide consent
2. Patients with intestinal tuberculosis, indeterminate colitis or uncharacterized bowel disorder.
3. Antibiotic therapy within the past month.

Diagnosis of ulcerative colitis was based on the following criteria (*Ouyang et al.*,)

1. History of diarrhea or rectal bleeding for 6 weeks or more and
2. Colonoscopy and histology showing features suggestive of ulcerative colitis

The extent of the disease was assessed by colonoscopy and biopsy and was classified as proctosigmoiditis (involvement of rectum and sigmoid colon), left sided colitis (involvement upto splenic flexure) and pancolitis (disease proximal to splenic flexure).

Diagnosis of Crohn's disease was based on the presence of following features in varying combination (*APDW 2004*)

1. Colonoscopy: Skip areas, cobblestone appearance, linear or serpiginous ulcer, fistula
2. Histopathology: Crypt architecture abnormalities, mononuclear infiltration, granulomas, transmural inflammation, segmental distribution of the lesion, patchy and focal inflammation.
3. Radiology: Skip lesions, fistula (entero-enteric or enterocutaneous), cobblestone appearance, combination of linear ulcer, deep transverse cleft, asymmetric involvement, creeping fat.
4. Response to therapy

Methods:

All patients included in the study were formally interviewed by the principal investigator who also performed a complete clinical examination. The nature of the disease was explained and investigations were then advised. These included upper and lower gastrointestinal endoscopy with segmental biopsies which is part of the current standard of care in this institution, and all investigations were done only as clinically indicated. Disease severity of CD was assessed using Harvey Bradshaw score (<4, mild; 5–8, moderate; >9, severe) and of UC using Montreal disease activity index [S0- Clinical Remission (asymptomatic); S1- Mild UC {passage of 4/fewer stools /day (with or without blood), absence of any systemic illness and normal inflammatory markers (ESR)}; S2- Moderate UC (more than 4 stools per day but with minimal signs of systemic toxicity) ; S3- Severe UC (at least 6 bloody stools daily, pulse rate of at least 90

beats/min, temperature of at least 37.5C, Hb <10.5g/100mL and ESR of at least 30mm/hr)]. Clinical details and laboratory results were all entered into structured forms and retained separately from the case records. The response to therapy and follow up were also recorded.

The presence of joint symptoms was noted. The pattern of joint involvement was noted and functional index was graded. The joint findings were cross-checked by a specialist rheumatologist. Radiographs of the involved joints were done for deformities. X rays of sacroiliac joint (antero posterior view) and spine were obtained and sacroiliac joint changes were graded according to New York criteria (*calin et al.*,).

Patients were also asked to provide a sample of stool to the laboratory for quantitative molecular characterization of specific classes of the bacterial flora - *Bacteroides*, *Eubacterium*, *Clostridium*, bifidobacteria, and lactobacilli. These bacterial groups were chosen for the following reasons. *Bacteroides* is generally believed to be aggressive in stimulating the innate immune system, while Bifidobacteria and Lactobacilli are both used as probiotics to ameliorate inflammation. Specific alterations in *Eubacterium* and certain classes of *Clostridia* are known to occur in inflammatory bowel disease. A separate control group was not included in the study because the intention was to compare the bacterial flora of patients with arthritis and the flora of patients without arthritis.

Written informed consent to enter the study was obtained from all participants. The protocol and consent form were approved by the Institutional Review Board comprising the scientific and ethical research committee.

Sample size calculation

This was a cross-sectional study of a population of individuals with inflammatory bowel disease. The sample size considerations were therefore as follows, and are different from sample sizes from a conventional case-control study. We assumed that 15% of the IBD patients would have joint disease. This study was powered on the basis of finding quantitative differences in the fecal bacterial flora. Utilising mean and standard deviation data from our previous studies of the fecal bacterial flora (*Balamurugan et al. Journal of Gastroenterology & Hepatology* 2008) we calculated that a sample size of 138 patients (i.e. 18 patients with joint symptoms and 120 without) would provide 80% study power to be able to discriminate patients with a two-fold change in specific bacterial population. In this context, it must be noted that traditional quantitative intestinal microbiology considered ten-fold difference in bacterial population as biologically significant, and therefore the ability to discriminate two-fold difference was more than sufficient for our purposes.

Statistical analysis:

Comparison between the UC and the CD group as well as the joint and the non-joint groups was done by Fischer's exact test for categorical variables and Mann-Whitney U test for continuous variables. IBD patients with joint features were treated as "cases" and IBD patients without the joint features were treated as the controls for the purpose of data analysis. For continuous data with normal distribution mean and standard deviation is reported. For continuous data without normal distribution the median and range is reported. A two tailed p value of ≤ 0.05 was considered significant. Mean and standard deviation were calculated. All analysis was performed in SPSS for Windows Version 11.

DNA Extraction and PCR:

Sample collection and processing

Subjects were provided with stool collection kits that included a stool box, tissue, napkins and a wooden spatula. Fecal specimens were collected in sterile containers. Immediately after receipt in the laboratory, samples were stored at -80°C .

DNA EXTRACTION AND PURIFICATION

Principle: The QIAamp DNA Stool Mini Kit provides fast and easy purification of total DNA from fresh or frozen stool samples. QIAamp purified DNA is ideal for reliable use in PCR and other downstream enzymatic reactions. The QIAamp DNA Stool Mini Kit is designed for rapid purification of total DNA from up to 220 mg of stool and is suitable for both fresh and frozen samples. A special protocol is provided for isolating DNA from larger amounts of stool. The procedure comprises the following steps:

- Lysis of stool samples in Buffer ASL.
- Adsorption of impurities to InhibitEX.
- Purification of DNA on QIAamp spin columns.

Procedure:

1. Weigh 180–220 mg stool in 2 ml microcentrifuge tube and place tube on ice.
2. Add 1.4 ml Buffer ASL to each stool sample. Vortex continuously for 1 min or until the stool sample is thoroughly homogenized.
3. Heat the suspension for 5 min at 95°C .
4. Vortex for 15 s and centrifuge sample at full speed for 1 min to pellet stool particles.

5. Pipette 1.2 ml of the supernatant into a new 2 ml microcentrifuge tube (not provided) and discard the pellet.
6. Add 1 InhibitEX tablet to each sample and vortex immediately and continuously for 1 min or until the tablet is completely suspended. Incubate suspension for 1 min at room temperature to allow inhibitors to adsorb to the InhibitEX matrix.
7. Centrifuge sample at full speed for 3 min to pellet inhibitors bound to InhibitEX.
8. Pipette all the supernatant into a new 1.5 ml microcentrifuge tube (not provided) and discard the pellet. Centrifuge the sample at full speed for 3 min.
9. Transfer of small quantities of pellet material from step 7 will not affect the procedure.
10. Pipette 15 μ l Proteinase K into a new 1.5 ml microcentrifuge tube.
11. Pipette 200 μ l supernatant from step 8 into the 1.5 ml microcentrifuge tube containing Proteinase K.
12. Add 200 μ l Buffer AL and vortex for 15 s.
13. Incubate at 70°C for 10 min.
14. Add 200 μ l of ethanol (96–100%) to the lysate, and mix by vortexing.
Optional: Centrifuge briefly to remove drops from the inside of the tube lid.
15. Label the lid of a new QIAamp spin column placed in a 2 ml collection tube.
16. Carefully apply the complete lysate from step 13 to the QIAamp spin column without moistening the rim. Close the cap and centrifuge at full speed for 1 min.

17. Place the QIAamp spin column in a new 2 ml collection tube, and discard the tube containing the filtrate.

18. Carefully open the QIAamp spin column and add 500 μ l Buffer AW1.

Centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the collection tube containing the filtrate.

19. Carefully open the QIAamp spin column and add 500 μ l Buffer AW2.

Centrifuge at full speed for 3 min. Discard the collection tube containing the filtrate.

20. Transfer the QIAamp spin column into a new, labeled 1.5 ml microcentrifuge tube (not provided) and pipette 200 μ l Buffer AE directly onto the QIAamp membrane.

21. Incubate for 1 min at room temperature, then centrifuge at full speed for 1 min to elute DNA.

22. DNA yield is determined by measuring the concentration of DNA in the eluate by its absorbance at 260 nm. Absorbance readings at 260 nm (A_{260}) should fall between 0.15 and 1.0 to be accurate.

Quantitative PCR

The molecular methods and protocols used for fecal bacterial quantitation have been standardized for this laboratory and published earlier (Balamurugan et al, JPGN 2008, JGH 2008, AJCN 2009). Real-time PCR primers were designed for bacterial species /groups, representing fecal microbes. The Ribosomal Database Project (Maidak et al., 2001; <http://rdp.cme.msu.edu/html/>) was utilized for identifying the primers that were likely to bind with the desired specificity to the rDNA of the selected target bacteria. The

PCR primers against *Bacteroides-Prevotella-Porphyromonas Bifidobacterium* genus *Lactobacillus*, *Desulfovibrio* specific primer and *Eubacterium rectale*-*Clostridium* coccoides cluster were designed on the basis of 16S rRNA gene sequences, available at the National Center for Biotechnology Information databases. A wide range of bacterial species was tested to confirm the specificity of the developed primers to target bacteria using conventional PCR. The *Bifidobacterium* genus detected all *Bifidobacterium* species, *Lactobacillus acidophilus* group primers detected *L. acidophilus*, *L. amylovorus*, *L. amylolyticus*, *L. crispatus*, *L. gasseri* and *L. johnsonii*. The other genus or group-specific primers detected all bacteria in that genus or cluster. Primers were also used to amplify a conserved 16S rDNA sequence present in all bacteria, the universal primer set.

The PCR amplification and optimal annealing temperatures of the PCR primers were initially optimized with gradient PCR in a Chromo 4 system (MJ Research/Biorad, USA) using SYBR Green master mix (Eurogentec, Belgium). All PCRs were performed in duplicate in a volume of 20µl, using high profile tubes and ultraclear sealing caps (MJ Research/Biorad, USA). The amplified products after a gradient PCR were subjected to electrophoresis and checked for the single, expected band and also were compared with the melting curve analysis. The following optimized PCR conditions were used for the real-time PCR. Reaction mixtures had 2X SYBR green master mix (200µM each dNTPs, 5mM MgCl₂), 0.5µM each primer, 2µl of template and water was used to make up to the final volume. The thermal cycling conditions started with an initial activation step at 50°C for 2 min and an initial denaturation step at 95°C for 10 min, which was followed by 45 cycles of denaturation at 95°C for 15 sec, annealing at 61°C for 30 sec, extension at

72°C for 30 sec and a plate read step. The final extension step at 72°C for 10 min was followed by a melting curve analysis, which was performed by increasing the temperature from 40°C to 95°C, with a raise in temperature by 1°C every 10 sec with a plate read step to read the fluorescent signal.

For quantitation of the number of bacterial for each target bacterial groups present in each sample, fluorescent signals detected from each sample and compared to a standard curve generated with standard DNA in the same experiment. Quantitation was analyzed by the Opticon 3.1 software provided with the Chromo 4 real-time PCR. Bacterial quantitation data were expressed as log₁₀ CFU/g stool.

RESULTS

A total of 128 patients could be recruited for the study during this period. Of the 128 patients with IBD, 60 patients had a diagnosis of CD (mean \pm SD age: 38.2 ± 15.3 yr; range from 12 yr to 70 yr) and 68 patients had UC (mean \pm SD age: 37.5 ± 14.6 yr; range from 2 yr to 78 yr). There were more men patients – UC (M:F = 38:30) and in CD (M:F = 40:20). Majority of the patients belonged to eastern (n=66) and southern (n=48) parts of India. The mean duration of delay in diagnosis was 66 ± 79.4 months.

Table : 1 Clinical manifestations of patients with inflammatory bowel disease:

CI Features	CD n =60 (47%)	UC n=68 (53%)	IBD n = 128 (100%)
Age, Yr	38.22 \pm 15.3	37.53 \pm 14.6	37.8 \pm 14.8
Sex M:F	40:20	38:30	78:50
Abdominal pain	49 (82%)	46 (67.6%)	95 (74.2%)
Diarrhoea*	33 (55%)	68 (100 %)	101 (79%)
Blood in stools*	28 (46.7%)	66 (97.1%)	94 (73.4%)
Fever*	22 (36.7%)	8 (11.8%)	30 (23.4%)
Intestinal Obstruction*	29 (48.3%)	2 (2.9%)	31 (24.2%)
Weight loss < 5 Kg:5-10: >10Kg wt loss Rest no weight loss	16:22:7 26.7%:36.7%:11.7%	32:12:4 47.1%:17.6%:5.9%	48:34:11 37.5%:26.6%:8.6%
Perianal features Fissure: Abscess: Fistula: Ulcer	5:1:6:1	6:1:3:0	11:2:9:1
Past h/o appendicectomy*	6 (10%)	0	6
Arthritis no. (%)	13 (21.6)	11 (16.1)	24 (18.7)

Values shown are mean \pm SD or number (percentage). * p < 0.05

Table 2: Laboratory parameters in the study population

	CD 60	UC 68
Hb [mean(SD)]; range	10.7 (2.4); 2.7 – 15.5	11(2.6) 4.5 – 16.9
MCV (mean)	76.2	77.2
ESR [mean(SD)]; range	37.8 (31.3); 2-135	39.1 (29)
CRP [mean(SD)]	18.3 (35.1)	24.9 (38.2)
S. Albumin [mean(SD)]; range	3.9 (0.8); 2-5	3.5 (1)
SAP [mean(SD)]	80 (57)	92.2 (46.6)
UGI : Not Done Normal Others Granuloma	14 40 Ulcer/erosion/nodule: 6 3 (5%)	38 27 Antral granularity/ attenuated D2 folds: 3 0
LGI: Not Done Findings: Granulomas	9 Ileal ulcer/ nodularity: 17 Skip lesions: 13 Deformed IC valve: 2 Fistula: 1 Diffuse ulceration: 2 Normal : 20 8	0 Proctitis: 16 (23.5%) Proctosigmoiditis: 20 (29.4%) Pancolitis: 32 (47.1%) 0
Radiology: BMFT CECT: USG Abdomen: Not Done	Possible CD: 8; Normal: 5 Possible CD: 18; Normal: 4 Possible CD: 1; Normal: 3 21	Normal: 11; UC: 2 Normal: 1; UC: 3 Normal: 5; UC: 2 44
Behaviour	Strictureing: 31 (51.7%) Non structuring – non fistulizing: 26 (43.3%) Fistulizing: 2 (3.3%) Strictureing & fistulizing: 1 (1.7%)	
Location	Colonic 11 (18.3%) Ileal 29 (48.4%) Ileocolonic 18 (30%) UGI 2 (3.3%)	Procto - Sigmoiditis : 16 (23.5%) Left sided colitis: 20 (29.4%) Pancolitis: 32 (47.1%)
Severity	<4: Mild: 24 (40%) 5-8: Moderate: 22(36.7%) >9: Severe: 14 (23.3%)	S0 :1 (1.5%) S1: 14 (20.6%) S2: 27 (39.7%) S3: 26 (38.2%)

Crohn's disease:

The mean age of patients with CD was 38.2 ± 15.3 years with majority being male patients (67%). Abdominal pain was the commonest symptom followed by tiredness and diarrhoea. Extra intestinal features were present in 14 patients – joint symptoms in 13 (with a patient having episcleritis) and a patient with scleritis alone. Ileal disease was the commonest type followed by ileocolonic and colonic disease. Disease severity at diagnosis assessed using the Harvey Bradshaw score showed the majority (59%) of patients had moderate to severe disease.

All patients were treated with 5-ASA preparations, the type of preparation (5 – amino salicylate, Sulfasalazine) chosen depending on site of disease. Remission induction using oral steroids was done in 17 (28.3%) patients. Remission was maintained with Mesacol – 57 (95%) along with oral steroids – 9 (15%) and azathioprine in 15 (25%) patients. Five patients required intravenous steroids for attaining remission and 3 received rectal steroid enema preparation for control of rectal symptoms. Nine (15%) were treated with steroids (Budesonide 3, Prednisolone 6) and 15 (25 %) steroid dependent / resistant patients with Azathioprine / Methotrexate. Seventeen patients (28.3 %) had surgery at diagnosis or during follow up for intractable symptoms or for severe disease /failure to attain remission (12 for stricture resection/ stricturoplasty and 5 had right hemicolectomy). Twenty four (40%) patients received empiric anti-tuberculous therapy in the past.

Ulcerative colitis:

The mean age of patients with UC was 37.6 ± 14.6 years with majority being males (56%). Diarrhoea was present in all patients while 46 (67.6 %) had abdominal pain. Extra intestinal manifestations in the form of scleritis/ episcleritis were present in only 4 (5.8 %) patients. One patient had pyoderma gangrenosum and 2 others had oral ulcers. Primary sclerosing cholangitis was noted in a patient. Thirty two (47.1 %) patients had pancolitis while 16 (23.5 %) had only proctosigmoiditis. Disease activity (Montreal) was moderate in 39.7 % patients, severe in 38.2 % and mild in 22.1 %. All patients were treated with 5-ASA except the patients who underwent total proctocolectomy – 3 (4.4%). For remission induction, 37 patients received oral steroids (Budesonide 3; rest with prednisolone) and in 7 patients intravenous steroids were used. Twenty one patients (30.9%) received azathioprine for maintenance of remission.

The clinical and laboratory profile of the two groups are shown in Table 1 & 2.

Joint symptoms in Inflammatory Bowel Disease:

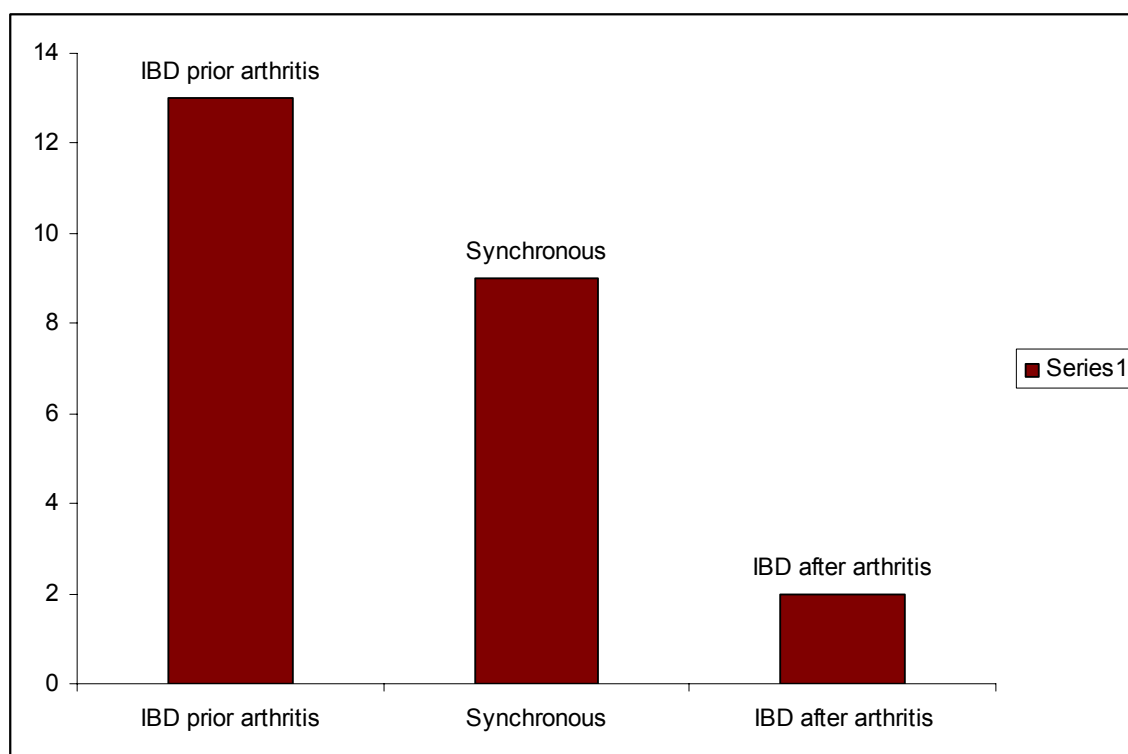
Arthritis occurred in twenty- four patients (18 %); 11 with UC (16.1%) and 13 with CD (21.6%). Nine (37%) had simultaneous joint & IBD symptoms; in 2 (8.3 %) patients joint symptoms were present before the appearance of IBD symptoms, and the remaining 13 (54.7%) had joint symptoms after the onset of IBD symptoms (Fig 3). It was therefore unusual for joint disease to precede manifestation of IBD symptoms. Both peripheral arthritis and sacroiliitis were found in five patients (3.9%). (Table 3).

Table : 3: Arthritis according to the extent of bowel involved and the type of arthritis in IBD.

IBD (n=128)	Crohn's disease (n=60)		Ulcerative colitis (n=68)		
	Colitis n = 29 (48.3%)	Ileitis n= 49 (81.7%)	Proctosigmoiditis n= 16 (23.5%)	Left sided colitis n= 20 (71.4%)	Pancolitis n=32 (47%)
With joint involvement no. (%) 24 (18.7)	9 (31)	4 (8.1)	0 (0)	2 (10)	9 (28)
Peripheral no. (%) 12 (9.3)	4 (13.7)	2 (4)	0 (0)	1 (5)	5 (15.6)
Mixed no. (%) 5 (3.9)	1 (3.4)	2 (4)	0 (0)	0 (0)	2 (6.2)
Axial no. (%) 7 (5.4)	4 (13.7)	0 (0)	0 (0)	1(5)	2 (6.2)

In CD patients, arthropathy was significantly more common in patients with colitis (9/29) compared to those with ileitis alone (4/49) (Chi square test, $P=0.0127$). In UC patients, arthropathy showed a trend towards increasing joint involvement with increasing degree of colonic involvement (proctitis 0/16, left sided colitis 2/20 and pancolitis 9/32) with the Chi-square test for trend being significant ($P=0.0271$).

Fig: 3: Onset of IBD with relation to arthritis. IBD usually preceded arthritis, and less commonly occurred simultaneously with arthritis.



Peripheral joint involvement:

Peripheral arthritis was found in twelve patients (9.3 % of total) and was not significantly different between UC (7/68) and CD (5/60). Eight of the 12 patients had colonic involvement (66.7%). Joint involvements tended to be monoarticular or oligoarticular. The most frequently affected joint was knee joint (14 patients) followed by ankle (7 patients), elbow (4 patients), hip (3 patients), shoulder and wrist in 2 patients each. Only one of the 12 (8.3%) patients had more than 6 joints involved. The baseline joint involvement is depicted in Figures 4 & 5.

Fig. 4: **Number of joints involved in IBD patients with peripheral arthritis**

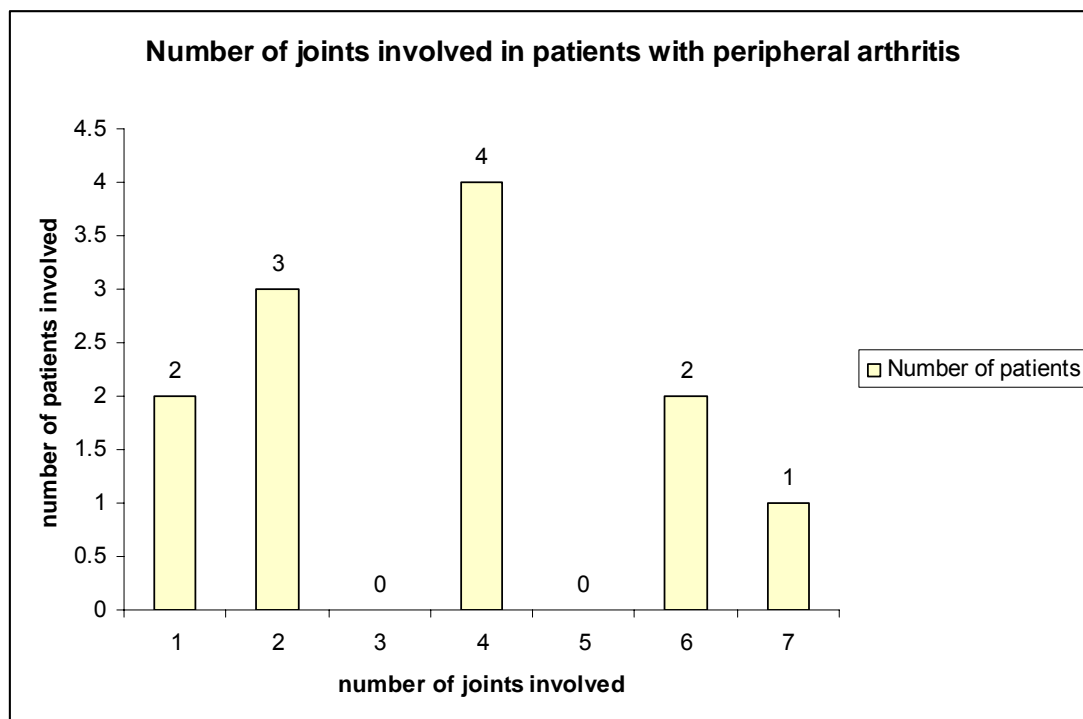
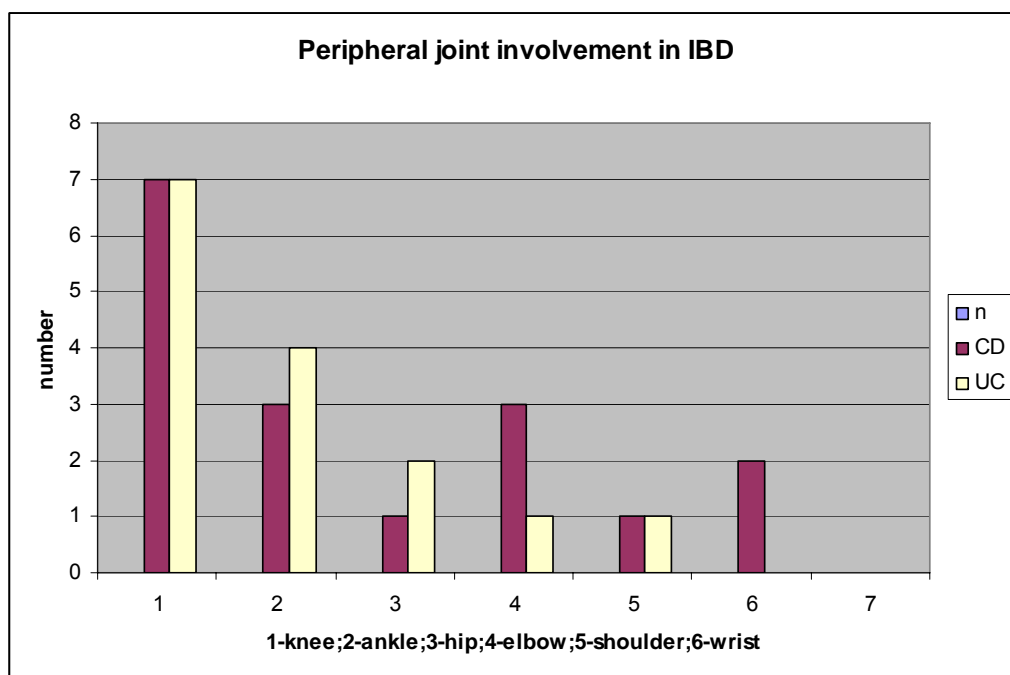


Fig 5: **Pattern of peripheral joint involvement in IBD.** Knee was most commonly involved, followed by ankle and elbow.



Axial joint involvement:

Radiographic sacroiliitis was observed in eight (3 UC and 5 CD) of the 24 IBD patients with joint symptoms (33.3%) patients. Spondyloarthropathy as described according to the ESSG criteria (*Gomariz et al*) was diagnosed in six (1 UC and 5 CD) patients. All except one patient had colonic involvement in the spondyloarthropathy group (83.4%). The remaining one patient had isolated small bowel involvement.

Joint symptoms in Ulcerative colitis:

Seven (10.2% of total) patients had peripheral arthritis, 3 (2.3% of total) had axial arthropathy and another 2 had features of both axial and peripheral arthritis. Three patients had radiological sacroiliitis (27.2%) and only one (9 %) had spondyloarthropathy. Nine of 32 UC patients (28%) with pancolitis had arthritis while none of the 16 patients with proctosigmoiditis had arthritis. On comparing patients with and without joint involvement, a higher CRP value, low serum albumin, low mean corpuscular volume & increased stool frequency were found in the UC patients with joint involvement (Table 6). The higher the value the more is the chance of having joint involvement. The duration of illness and mean duration of symptoms prior to the diagnosis were not statistically significant between the 2 groups. The important baseline characteristics between the 2 groups are as follows (table 4):

Table: 4 : Clinical characteristics of the patients with arthritis in ulcerative colitis:

Total UC patients n= 68 (100%)	With arthritis n = 11 (16.1%)	Without arthritis n = 57 (83.9%)	P value
Age in Yr; mean \pm SD	34 \pm 14	38 \pm 14.6	NS
Sex M:F	6:5	32:25	NS
Smoking Yes: No	1:10	1:56	NS
Stool Frequency * mean \pm SD	8.6 \pm 3.4	5.3 \pm 3.4	p < 0.05
Mean corpuscular volume*	67 \pm 23.7	78 \pm 15	p < 0.05
C - reactive protein*	45.1 \pm 64.9	12.8 \pm 22.4	p < 0.05
Serum albumin*	3.3 \pm 0.95	4 \pm 0.77	p < 0.05
Hemoglobin	10.9 \pm 2.1	11 \pm 2.7	NS

Values shown are absolute numbers or mean \pm SD.

Joint symptoms in Crohn's disease:

In CD, arthritis occurred in 39% of the patients with colonic involvement (9 had arthritis of total of 29 with colonic involvement) but in only 4 of 49 CD patients with small bowel involvement had arthritic symptoms (8.2%). This was statistically significant (P=0.0127). Spondyloarthropathy was noted in 4 (6.6%) of the CD patients while peripheral arthritis was noted in 6 (10%) patients. Four patients had features of both axial and peripheral arthritis. On cross tabulating using Mann Whitneys test and Fischers exact t test, elevated

CRP was the only parameter found to be significantly correlated with the joint involvement. Low serum albumin, high ESR and greater height were approaching significance though are found to be not significant statistically.

Table: 5: Clinical characteristics of the patients with arthritis in Crohns Disease:

Total CD patients n= 60 (100%)	With arthritis n = 13 (21.6 %)	Without arthritis n = 47 (78.4%)	P value
Age in Yr; mean \pm SD	36.9 \pm 9.6	38.5 \pm 16.6	NS
Sex M:F	9:4	31:16	NS
Height in cms	156.4 \pm 10.3	153.5 \pm 8.6	NS
Abdominal pain yes: no	10 :3	39:8	NS
Diarrhoea	8:5	25 :22	NS
Smoking Yes: No	0: 13	5:42	NS
Hemoglobin mean \pm S.D	10.8 \pm 1.3	10.6 \pm 2.7	NS
Mean corpuscular volume	77.2 \pm 10	77.2 \pm 21	NS
C reactive protein*	62 \pm 56.5	14.6 \pm 23.4	0.0047
Serum albumin	3.3 \pm 1	3.6 \pm 1.1	NS
Erythrocyte sedimentation rate	44 \pm 27	37.7 \pm 29.7	NS

* p <0.05 using Mann-Whitney test

Real time PCR analysis:

Average bacterial quantifications in each patient group are summarized in Table 6. The results were then compared to IBD with joint and IBD without joint groups (Fig. 1)

Table 6: Fecal microbial flora in IBD patients with and without joint disease

Log₁₀ values (mean \pm SD) are shown.:

Bacteria	IBD Without Joints	IBD with Joints	P Value
Total Bacteria	9.2 \pm 0.071	9.3 \pm 0.110	0.5371
Enterobacteriaceae	8.1 \pm 0.110	8.6 \pm 0.260	0.0772
<i>Bifidobacterium</i> genus	8.2 \pm 0.130	8.3 \pm 0.220	0.8734
<i>Clostridium coccoides</i>	7.102 \pm 0.1767	6.557 \pm 0.3796	0.1067
<i>Bacteroidetes</i>	9.2 \pm 0.150	9.2 \pm 0.280	0.9658
<i>Lactobacillus</i> group	5.748 \pm 0.1301	5.888 \pm 0.1841	0.6383
<i>Desulfovibrio</i> genus	6.632 \pm 0.1024	6.539 \pm 0.1607	0.8545

The most prevalent phyla were *Bacteroidetes* and *Bifidobacterium* in both groups and no commensal was significantly different between the 2 groups. Quantitative molecular characterization of specific classes of the bacterial flora - *Bacteroides*, *Eubacterium*, *Clostridium*, bifidobacteria, and lactobacilli revealed no difference of flora between the IBD patients with and without joint involvement.

Table 7. IBD: Fecal microbial flora in UC compared to CD patients.

Log₁₀ values (mean \pm SD) are shown.

Bacteria	CD	UC	P Value
Total Bacteria	9.2 \pm 0.09	9.39 \pm 0.10	0.21
Enterobacteriaceae	8.11 \pm 0.16	8.53 \pm 0.17	0.05
<i>Bifidobacterium</i> genus	8.23 \pm 0.19	8.29 \pm 0.17	0.74
<i>Eubacterium</i>	6.88 \pm 0.24	7.26 \pm 0.23	0.20
<i>Bacteroides</i>	9.13 \pm 0.19	9.53 \pm 0.22	0.20
<i>Lactobacillus</i> group	5.92 \pm 0.17	5.82 \pm 0.19	0.89
<i>Desulfovibrio</i> genus	6.68 \pm 0.16	6.73 \pm 0.14	0.70

Between the two CD and UC groups, Enterobacteriaceae was noted to be more prevalent in UC than in CD (P<0.05). Rest of the commensals were similar between the 2 groups.

In subgroup analysis of CD by joint involvement there was no commensal identifying the propensity for joint involvement (Table 8).

Table 8. CD: Fecal microbial flora in CD patients with and without joint involvement. Log₁₀ values (mean \pm SD) are shown.

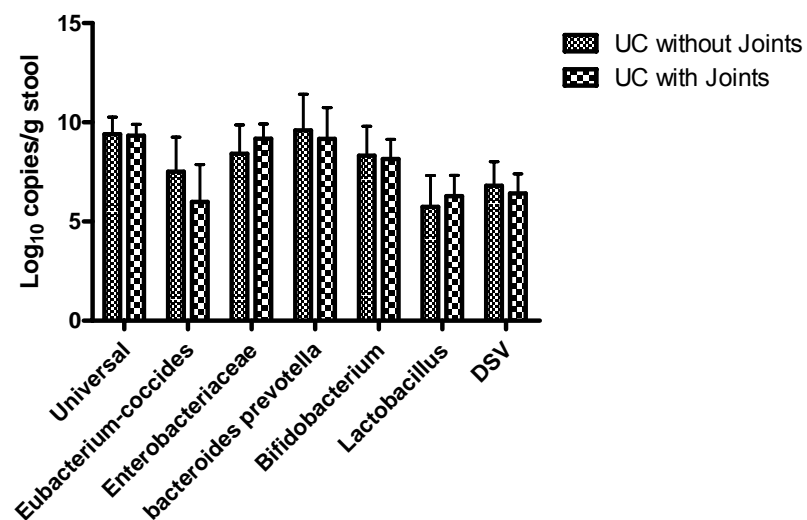
Bacteria	CD Without Joints	CD with Joints	P Value
Total Bacteria	9.16 \pm 0.120	9.34 \pm 0.13	0.23
Enterobacteriaceae	6.85 \pm 0.280	6.99 \pm 0.47	0.95
<i>Bifidobacterium</i> genus	8.18 \pm 0.220	8.45 \pm 0.30	0.6677
<i>Clostridium coccoides</i> groups	7.102 \pm 0.1767	6.557 \pm 0.3796	0.2029
<i>Bacteroidetes</i>	9.2 \pm 0.150	9.2 \pm 0.280	0.9462
<i>Lactobacillus</i> group	5.748 \pm 0.1301	5.888 \pm 0.1841	0.5377
<i>Desulfovibrio</i> genus	6.632 \pm 0.1024	6.539 \pm 0.1607	0.6269

Table: 9: UC: Fecal microbial flora in UC patients with and without joints Log₁₀ values (mean \pm SD) are shown.

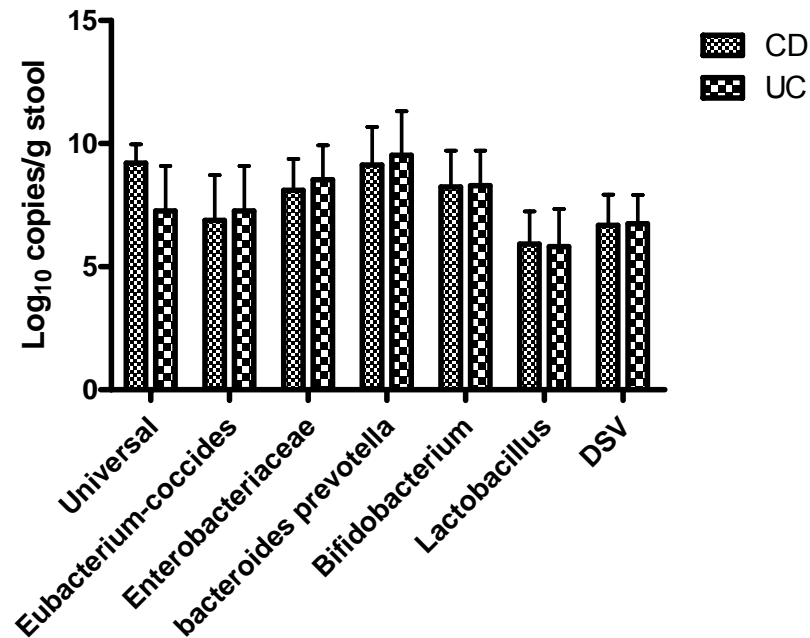
Bacteria	UC Without Joints	UC with Joints	P Value
Total Bacteria	9.408 \pm 0.1172	9.336 \pm 0.1810	0.9337
<i>Clostridium coccoides</i> groups	7.516 \pm 0.2452	5.993 \pm 0.5927	0.0108
Enterobacteriaceae	8.413 \pm 0.1984	9.175 \pm 0.2339	0.0410
<i>Bacteroidetes</i>	9.599 \pm 0.2479	9.168 \pm 0.4994	0.4881
<i>Bifidobacterium</i> genus	8.322 \pm 0.2026	8.151 \pm 0.3141	0.4881
<i>Lactobacillus</i> group	5.738 \pm 0.2178	6.282 \pm 0.3325	0.1475
<i>Desulfovibrio</i> genus	6.800 \pm 0.1667	6.413 \pm 0.3136	0.4924

On comparison of bacterial commensals in stools of patients of ulcerative colitis with and without joint disease, Enterobacteriaceae were found in a higher number in joint group and *Clostridium coccoides* were found in higher number in the group without joint involvement and were statistically significant ($p < 0.05$).

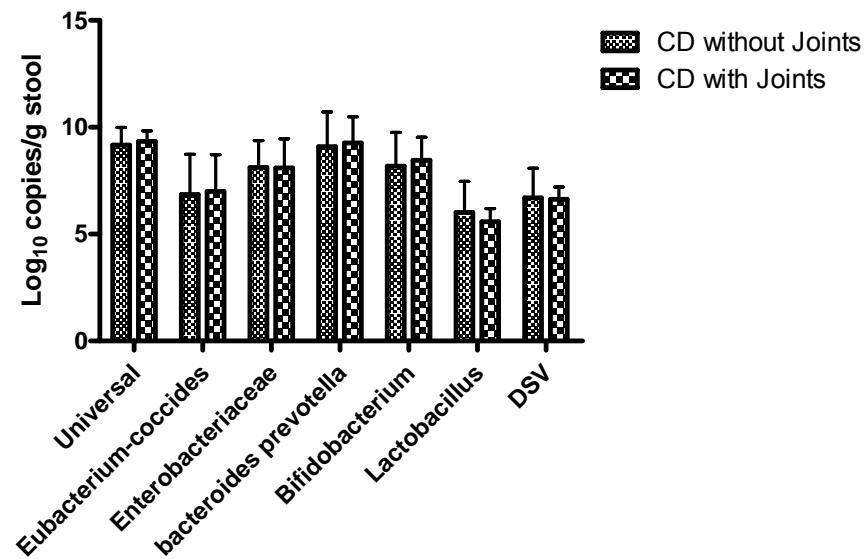
UC With and without joints: Quantification of dominant and subdominant bacteria in the fecal microbiota



CD Vs UC - Quantification of dominant and subdominant bacteria in the fecal microbiota



CD With and without Joints: Quantification of dominant and subdominant bacteria in the fecal microbiota



Disability following joint involvement in IBD:

Nine of the 24 patients had active joint complaints at the time of examination and the rest of the 16 had joint symptoms in the immediate 5 years of follow up. Five of 16 patients had severe joint involvement with restriction which resolved with therapy. One of them also had avascular necrosis of the hip (steroid related) which needed a total hip replacement.

The examined patients had normal chest wall expansion at 4th intercostal space as well as occiput to wall distance. Four of them had Achilles enthesitis. Mean PGA (patients general assessment of the activity) of joints in the past 1 week was 40 with a range from 10 to 80 on a 100 point scale with minimum score means less disease activity. The Bath ankylosing spondylitis global score (BAS-G) which denotes global health in the past 1 week to 6 months in a 10 point scale (maximum is severe disease activity) of the involved patients was 6.5. The Bath ankylosing spondylitis disease activity index in past 1 week (BASDAI) which comprises of 6 questionnaire each in a 10 point scale and the Bath ankylosing functional index in past 1 month (BASFI) comprising 10 activity questions in 10 point scale with the maximum being severe disease activity and both had a mean value of 2.5 out of 10 in the study group. The Bath ankylosing spondylitis metrology index (BASMI) which includes objective involvement of axial joints in a 0 – 10 point scale with 10 being maximum restriction revealed none of the patients had a score of more than one. The subjective component of joint activity was higher than the objective involvement in the study group.

For the peripheral joints, disease activity score (DAS) with the involved joints assessed for tenderness, limitation and swelling revealed 15 joints to be involved.

Arthralgias and non deforming arthritis were noted in 4 each and none had deforming arthritis.

Discussion

Ever since the first description of joint involvement with UC and CD in 1895 and 1930, several reports were published on this concept. The inter link between the gut and joint needs to be studied for determining the pathophysiology between the two diseases. The current opinion favors the concept of enteric organisms acting as bowel triggers in a disease susceptible individual (*Jan tore gran et al*).

To evaluate the prevalence of arthritis and the bacterial flora associated with IBD in India, we prospectively studied one hundred and twenty-eight patients with IBD, 68 with ulcerative colitis (UC) and 60 with Crohn's disease (CD). The clinical characteristics of arthritic flares are similar in both UC and CD. Palumbo et al reviewed the articular manifestations of 121 patients with IBD and found no distinguishing characteristics between the arthritis that is seen in CD and that seen in UC. (*Gravallese et al*). In our study arthritis was noted in twenty- four IBD patients (18 %); 13 with CD (21.6%) and in 11 with UC (16.1%). Thirteen (54.7%) patients had joint symptoms after the onset of IBD symptoms which correlate with the previous studies from other countries (*Gravallese et al*).

The pattern of joint involvement was common in both UC and CD groups. Peripheral arthritis alone was noted in 12 (9.3%) patients, peripheral arthritis along with axial involvement in 5 (3.9%), and axial involvement alone in 7 (5.4%) patients which

were similar to the review of prevalence of joint symptoms in IBD patients by Jan tore gran et al.

Arthritis in CD patients was associated significantly more commonly with colonic disease (9/29) compared to ileal disease (4/49). Arthritis in UC patients showed an association with increasing length of colonic involvement, being 0% in proctitis, 10 % in left sided colitis and 28 % in pancolitis. The most frequently affected peripheral joint was knee joint (14 patients) followed by ankle (7 patients), The most frequently affected joint was knee joint (14 patients) followed by ankle (7 patients) and radiographic sacroiliitis was observed in eight of the 24 IBD patients. The bacterial answer for this pattern of involvement was proposed in earlier reviews as the possibility of diseased bowel providing the immunological stimulus for the occurrence of arthritis along with higher bacterial loads in the distal gut. (*Gravallese et al*). Severe and extensive forms of UC correlated with more complicated joint involvement. In UC patients with pancolitis, arthritis and polyarthritis were observed significantly more often as noted by Dorofeyev et al.. Similar finding was observed in our study.

In UC, joint involvement was associated with lower albumin, higher ESR, higher CRP, increasing stool number, all indicating increased disease activity. In CD, joint involvement was associated with higher CRP, but not with any other index of disease activity. The sub group analysis was not done as the study was not powered adequately for sub-group analysis.

IBD is caused by a genetically determined defect in the down-regulation of inflammation driven by ubiquitous antigens. Luminal anaerobic bacterial antigens are the stimuli in Crohn's disease, but ulcerative colitis may also be caused by functionally abnormal

aerobic bacteria or primary defects in epithelial cell physiology (*Sartor et al*). In 90 (94.7%) UC patients with joint EIM, two or more types of facultative bacteria occurred. In patients with arthritis, bacterial associations such as *Staphylococcus-Klebsiella*, *Staphylococcus-Proteus* or *Staphylococcus-Klebsiella-Proteus* were observed more often. In all patients with joint manifestations, these facultative flora quantities were significantly higher than in the healthy population and UC patients without EIM (*A.E. Dorofeyev et al*). In our study we studied the commensals alone IBD with and without joint involvement and found that enterobacteriaceae were higher ($P < 0.05$ by Mann whitneys) in IBD with joint symptoms. Rest of the commensals studied was common between the 2 groups.

The subjective component of axial joint activity (BAS – G; BASDAI) was higher than the objective involvement (BASMI, BASFI) in the study group. The peripheral arthritis is migratory and non deforming and distinct from the peripheral arthritis is an arthritis involving large joints seen specifically in patients with spondylitis. The hips and shoulders are specifically involved and to a lesser extent the knees. Hips and shoulders may be permanently damaged as noted in a single patient with hip replacement. (*Gravallese et al*)

In UC, joint involvement was associated with lower albumin, higher ESR, higher CRP, increasing stool number, all indicating increased disease activity. In CD, joint involvement was associated with higher CRP, but not with any other index of disease activity.

In a study of 319 UC patients from Ukraine, bacterial flora was compared between the patients with and without joint involvement. Patients with joint EIM, severe dysbiosis with a facultative bacteria association were found. *Staphylococcus*, *Klebsiella* and *Proteus* were more commonly identified in patients with arthritis (Dorofeyev et al.). In our study we did not study the other facultative organisms. The study was designed to observe the pattern of commensals in joint involvement in Indian IBD patients. It is now commonly considered that the commensal flora of the intestine falls into three categories – those bacterial classes or groups that are aggressive (including *Bacteroides*, *Helicobacter* species, *Enterococcus fecalis*, and pathogenic *Escherichia coli*; those bacterial groups that are protective including *Lactobacillus* and *Bifidobacterium* species (which are used as “probiotics”) and those that are neutral which include the majority of *Escherichia coli* (Glenn Gibson et al). In this study, differences were noted in the quantitative bacterial flora between UC patients with and without arthritis, pointing to a possible association between such bacterial shifts (dysbiosis) and arthritis. On comparison of bacterial commensals in stools of patients of ulcerative colitis between joint and without joint groups, enterobacteriaceae were found in a higher number in patients with joint involvement whereas *Clostridium coccoides* were found in higher number in the group without joint involvement and were statistically significant ($p < 0.05$). Enterobacteriaceae may be deleterious to the human host if they get out of balance. Similarly, reductions in level of *Clostridium coccoides* may be associated with greater inflammation. This cluster of bacteria ferments carbohydrates to short chain fatty acids, including butyrate. These short chain fatty acids provide fuel energy to the colonic epithelium, but importantly they also ameliorate inflammation through suppression of

HSP70-dependent innate immune pathways. Short chain fatty acids such as butyrate also reduce colonic permeability thereby reducing the non-specific absorption of antigens from the intestine. In subgroup analysis of CD by joint involvement there was no commensal identifying the propensity for joint involvement. Between the two CD and UC groups, Enterobacteriaceae was noted to be more prevalent in UC than in CD ($P<0.05$). Rest of the commensals were similar between the 2 groups. The most prevalent phyla were Bacteroidetes and Bifidobacterium in both groups and no commensal was significantly different between the 2 groups. Quantitative molecular characterization of specific classes of the bacterial flora - *Bacteroides*, *Eubacterium*, *Clostridium*, bifidobacteria, and lactobacilli revealed no difference of flora between the IBD patients with and without joint involvement.

Conclusions

- This is the largest study to date, comparing the bacterial flora in stool in inflammatory bowel disease (IBD) patients with and without joint involvement.
- Prospective study of one hundred and twenty-eight patients with IBD, 68 with ulcerative colitis (UC) and 60 with Crohn's disease (CD).
- CD patients had abdominal pain (82%) as the commonest presentation followed by diarrhoea (55%). Diarrhoea (100%) was the commonest presenting symptom among UC patients followed by blood in stool (97%).
- Presence of abdominal pain, diarrhoea, blood in stools, fever, intestinal obstruction and past history of appendicectomy significantly differentiated between the CD and UC groups.
- Disease activity (Montreal) was moderate in 39.7 % patients, severe in 38.2 % and mild in 22.1 % in UC patients. In CD patients' disease severity as assessed using the Harvey Bradshaw score showed the majority (59%) of patients had moderate to severe disease.
- Arthritis was noted in twenty- four IBD patients (18 %); 13 with CD (21.6%) and in 11 with UC (16.1%). Arthritis was equally common in CD and UC groups. Thirteen (54.7%) patients had joint symptoms after the onset of IBD symptoms
- Peripheral arthritis alone was noted in 12 patients, peripheral arthritis + axial involvement in 5, and axial involvement alone in 7 patients

- Arthritis in CD patients was associated significantly more commonly with colonic disease (9/29 - 31%) compared to ileal disease (4/49 - 8.1%).
- Arthritis in UC patients showed a trend for association with increasing length of colonic involvement, being 0% in proctitis, 10% (2 of 20 patients) in left sided colitis and 28% (9 of 32 patients) in pancolitis.
- The subjective component of axial joint activity (BAS – G; BASDAI) was higher than the objective involvement (BASMI, BASFI) in the study group. The most frequently affected joint was knee joint (14 patients) followed by ankle (7 patients) and radiographic sacroiliitis was observed in eight of the 24 IBD patients
- In UC, joint involvement was associated with lower albumin, higher ESR, higher CRP, increasing stool number, all indicating increased disease activity.
- In CD, joint involvement was associated with higher CRP, but not with any other index of disease activity.
- The most prevalent phyla were *Bacteroidetes* and *Bifidobacterium* in both groups. Enterobacteriaceae numbers were slightly (but significantly) higher in patients with UC than in CD. In UC patients, joint involvement was associated with higher *Enterococcus* species and lower *Clostridium coccoides* cluster, suggesting a dysbiosis in these patients.

<u>PROFORMA FOR JOINT SYMPTOMS IN IBD</u>
--

Patients Name:

CMCH No:

Contact Address:

Occupation:

Income/month:

Telephone:

E Mail:

1. Age in years:

2. Year of birth:

3. Gender:

4. Mother Tongue: 1.Tamil 2.Telugu 3.Malayalam 4.Kannada

5.Bengali 6.Oriya 7.Hindi 8.Others

5. State Of Origin:

6. Weight (Kg):

7. Height (cm):

8. Mid arm circumference:

9. Religion: 1.Hindu 2. Muslim 3. Christian 4.Other

10. Education: 1. None 2. I- V Std 3. VI-X Std 4.Plus 2

5. Graduate

6. Post Graduate

Patient History:

1. At what age did the symptoms appear?

2. At what age was the disease diagnosed?

3. Which year was diagnosis first made?

4. Where was the diagnosis made? 1.CMC 2.Local hospital 3.Other

Have you ever had the following symptoms in relation to your illness?

5. Abdominal Pain 1.Yes 2.No

6. Diarrhoea 1.Yes 2.No

7. Blood in Stools 1.Yes 2.No

8. Urgency 1.Yes 2.No

9. Incontinence 1.Yes 2.No

10. Tiredness 1.Yes 2.No

11. Fever 1.Yes 2.No

12. Intestinal Obstruction 1.Yes 2.No

13. Have you lost weight? 1. Nil 2. <5Kg 3. 5-10Kg 4.>10Kg

14. What is the frequency of your stools now?

15. Have you had anal canal lesions: 1. Fissure 2. Ulcers 3.Fistulae

16. Have you had painful boils in legs/body: 1.Yes 2.No

17. Have you had painful redness of eyes: 1.yes 2.No

18. Any other problem:

Past Medical History & Family and Personal History:

19. Diabetes mellitus 1. Yes 2.No

20. Hypertension 1.Yes 2.No

21. Pulmonary tuberculosis 1. Yes 2.No

22. Extra Intestinal tuberculosis 1.Yes 2.No

23. Were you ever diagnosed to have intestinal TB? 1. Yes 2.No

24. Have you had appendectomy? 1. Yes 2.No
25. If yes, was it done for acute abdominal pain? 1. Yes 2.No
26. Have you had any other operation on abdomen? 1. Yes 2.No
27. Has any other family member had IBD? 1. Yes 2.No
28. Do you currently smoke cigarettes/beedies?
1. No 2. <7 per week 3. 7/wk to 10/day 4.> 10/day
29. In the past did you smoke cigarettes/beedies?
1. No 2. <7 per week 3. 7/wk to 10/day 4.> 10/day
30. Did you chew tobacco alone or with paan? 1. Yes 2.No
31. Do you take alcohol drinks?
1. Teetotaler 2. Social drinker 3.Moderate drinker 4.Heavy drinker
32. What diet do you take?
1. Strict Vegetarian 2.Eggs also 3.Non-vegetarian

Medical treatment:

33. Have you ever used aminosalicylates in the past? 1. Yes 2.No
34. Have you ever used oral steroids in the past? 1. Yes 2.No
35. Have you ever used rectal steroids in the past? 1. Yes 2. No
36. Have you ever used IV steroids in the past? 1.Yes 2.No
37. Are you currently using aminosalicylates? 1.yes 2.no 3.cannot say
38. Are you currently using steroids (any form)? 1.yes 2.no 3.cannot say
39. Are you currently using azathioprine/immunosuppressant?
- 1.yes 2.no 3.cannot say

40. Have you received drugs for TB in the past? 1.yes 2.no 3.cannot say

41. Are you currently taking drugs for TB? 1.yes 2.no 3.cannot say

42. Joint symptoms onset with IBD?

1. Simultaneous 2.Before GI symptoms 3. After GI symptoms

43. Relation with the activity of GI symptoms with Joint symptoms?

1. Abdominal pain 2.Diarrhoea 3.Blood in stools 4. Perianal disease

44.Laboratory findings:

Test	Date/Finding	Report/Biopsy
Hb (g/dl)		
MCV (fL)		
ESR (mm/hr)		
CRP		
S Albumin		
S Alk.Phos		
Stool Parasites x 2		
Endoscopy		
Colonoscopy		
BMFT/CECT Abdomen		
X Ray of joints		
Sacroileitis		
RA/HLA B27/ASCA		
Bowel Flora		
NOD 2 Mutation		

Date Of assessment: 45. Montreal classification for CD:

Age at diagnosis: radiology/endoscopy/pathology/surgery

A1: Below 16 years A2: Between 17 - 40 years A3: Above 40 yrs

Maximum extent of the disease: small and large bowel examination needed:

L1: Ileal (no colon) L2: Colonic L3: Ileocolonic

L4: Isolated upper disease (L4 –Modifier – Be added to L1-L3 when concomitant upper gastrointestinal disease is present)

Behaviour:

B1: Non- Stricturing, non-penetrating B2: Stricturing (obstructive)

B3: Penetrating (fistula, mass, abscesses, ulcers)

P: Added to B1-B3 when concomitant perianal disease is present

Severity for CD: Harvey Bradshaw Index (HBI, Simple Index)

1 General well being (0: very well, 1: slightly below par, 2: poor, 3: very poor, 4: terrible)

2 Abdominal pain (0: none, 1: mild, 2: moderate, 3: severe)

3 Number of liquid stools daily:

4 Abdominal mass (0: none, 1: dubious, 2: definite, 3: definite and tender)

5 Complications: arthralgia, uveitis, erythema nodosum, aphthous ulcer, pyoderma gangrenosum, anal fissure, new fistula, Abscess (score 1 per item)

46. Total HBI score: HBI Score: (<4, mild; 5–8, moderate; >9, severe)

Date Of assessment:

45.Montreal Classification of Extent of Ulcerative colitis

E1(Procto - Sigmoiditis): Ulcerative Proctitis: Involvement limited to rectum (proximal extent of inflammation is distal to the recto sigmoid junction

E2: Left Sided UC (Distal UC): Involvement limited to a proportion of the colorectum distal to the splenic flexure

E3: Extensive UC (Pan Colitis \pm back wash Ileitis): Involvement extends proximal to the splenic flexure

46.Montreal Classification of severity of Ulcerative Colitis

S0- Clinical Remission- Asymptomatic

S1- Mild UC-Passage of 4/fewer stools /day (with or without blood), absence of any systemic illness and normal inflammatory markers (ESR)

S2- Moderate UC- More than 4 stools per day but with minimal signs of systemic toxicity

S3- Severe UC-At least 6 bloody stools daily, pulse rate of at least 90 beats/min, temperature of at least 37.5C,Hb <10.5g/100mL and ESR of at least 30mm/hr

Musculo Skeletal Manifestations

IBD Symptoms Duration:

Joint symptoms Duration

Chest wall expansion at 4th intercostals space:

Occiput to wall distance:

Enthesitis:

Achilles tendinitis: Pain/difficulty in walking/swelling/tenderness for 1 month

Plantar fasciitis: Pain/difficulty in walking/swelling/tenderness for 1 month

Dactylitis: sausage digit: + / -

Peripheral Arthritis: 1/more painful/painfully restricted swollen joints for at least 3 months

ESSG entry criteria for SpA: 1 OR 2

- Inflammatory back pain — Inflammatory If 4 OF 5 : (walk at night for pain)
 - Age: < 40 years + / -
 - Onset: Insidious + / -
 - 3 months Duration + / -
 - Morning Stiffness + / -
 - Improvement with exercise + / -
- Asymmetrical synovitis — Predominantly lower limbs

Warmth/difficulty in walking/swelling/reduction in both active and passive range of motion Worse after a period of rest + / -

Additional criteria — any one of the above is positive,

- Positive family history of psoriasis/ankylosing spondylitis
- Psoriasis
- Inflammatory bowel disease
- Urethritis, cervicitis, or acute diarrhea within one month before arthritis
- Buttock pain alternating between buttocks
- Enthesopathy
- Plain film radiographic evidence of sacroileitis

* **GENERAL HEALTH** or patients' global assessment of disease activity:
How active your arthritis been during the last 7 days?
(PATIENTS ASSESSMENT IN MM)

NO ACTIVITY -----→ HIGHEST ACTIVITY POSSIBLE

0 ----- 10 ----- 20 ----- 30 ----- 40 ----- 50 ----- 60 ----- 70 ----- 80 ----- 90 ----- 100

AXIAL JOINTS : BAS-G; BASDAI ; BASFI ; BASMI

The Bath Ankylosing Spondylitis Global Score (BAS –G)

		Total/10
1	How have you been over the last week? VERY GOOD -----VERY BAD	
2	How have you been over the last six months? VERY GOOD -----VERY BAD	
	TOTAL OUT OF 20	
	TOTAL / 2 (BAS-G SCORE)	

BASDAI:

- A. If you are currently taking medication for your AS, please give the name and dose that is on the bottle/packet.
- B. Please mark on the line below to indicate the effectiveness of the medication in relieving your symptoms
NO EFFECT ----- VERY EFFECTIVE

THE BATH ANKYLOSING SPONDYLITIS DISEASE ACTIVITY INDEX (BASDAI)

Please draw a mark on each line below to indicate the level of ability with each of the following activities during the PAST WEEK(0-10 points each)

1	How do you describe the overall level of fatigue/tiredness you have experienced? NONE ----- VERY SEVERE	
2	How would you describe the overall level of AS neck, back or hip pain you have had? NONE ----- VERY SEVERE	
3	How would you describe the overall level of pain/swelling in joints other than neck, Back or hips you have had? NONE ----- VERY SEVERE	
4	How do you describe the overall level of discomfort you have had from any areas tender to touch or pressure? NONE ----- VERY SEVERE	
5	How do you describe the overall level of discomfort you have had from the time you wake up? NONE ----- VERY SEVERE	
6	How long does your morning stiffness last from the time you wake up? 0 ----- 1/2 ----- 1 ----- 1 1/2 ----- 2 or more hours	
	MEAN OF 5&6	
	TOTAL OF 1 TO 4 ADDED TO MEAN OF 5&6 (TOTAL OUT OF 50)	
	TOTAL / 5 (BASDAI SCORE)	

THE BATH ANKYLOSING SPONDYLITIS FUNCTIONAL INDEX (BASFI)

Please draw a mark on each line below to indicate the level of ability with each of the following activities during the LAST MONTH (0-10 points each)

1	Putting on your socks or tights without help or aids? Easy-----Impossible	
2	Bending forward from the waist to pick up a pen from the floor without an aid? Easy-----Impossible	
3	Reaching up to a high shelf without help or aids? Easy-----Impossible	
4	Getting out of an arm less dining chair without using your hands or any help? Easy-----Impossible	
5	Getting off the floor – without help –from lying on your back? Easy-----Impossible	
6	Standing unsupported for 10 minutes without discomfort? Easy-----Impossible	
7	Climbing 12-15 steps without using the hand rails or walking aids? Easy-----Impossible	
8	Looking over your shoulder without turning your body? Easy-----Impossible	
9	Doing physically demanding activities (e.g., physio exercises) Easy-----Impossible	
10	Doing a full day activities at home or at work? Easy-----Impossible	
	TOTAL OUT OF 100	
	TOTAL/10 (BASFI SCORE)	

THE BATH AS METROLOGY INDEX (BASMI)

Measure * Mean of right and left	Mild 0	Moderate 1	Severe 2	Patients score
*Lumbar side flexion Stand to a wall, feet 30cm apart, hands by side, tip of middle finger to the floor & side flexion and measure	>10 cm	5-10cm	<5 cm	
Lumbar flexion (modified Schober) Feet 30 cm apart, line midway between iliac crests, a point 10cm above and another 5 cm below – gap of 15cm; Flexion and measure – increase is noted	>4 cm	2- 4 cm	<2 cm	
*Tragus to wall distance Back to wall, feet 30 cm apart, side of face to wall and the distance of tragus to wall	<15 cm	15-30 cm	>30 cm	
Intermalleolar distance Supine, knees extended, legs apart as far as possible, distance between the medial malleoli	>100 cm	70 – 100cm	<70 cm	
*Cervical Rotation Supine, Forehead in horizontal position, head rotation as far as feasible and goniometry	> 70 cm	20 – 70 cm	<20 cm	
Patient BASMI score - Total				

PERIPHERAL JOINTS

DISEASE ACTIVITY SCORE - JOINT COUNT

Joint		Right			Left	
	Tenderness	Swelling	Limitation	Tenderness	Swelling	Limitation
Shoulder						
Elbow						
Wrist						
MCP						
PIP						
DIP						
Hip						
Knee						
Ankle						
MTP						
PIP						
DIP						
Total						

JOINT COUNT: Axial:

Joint	Tenderness	Swelling	Limitation
Cervical			
Temporo mandibular			
Sternoclavicular			
Acromioclavicular			
Total			

PATIENT'S INFORMATION SHEET AND CONSENT

Study title: Joint manifestations in IBD patients and the relation of the bowel flora

Principal Investigator: Dr. Avinash Balekuduru

INFORMATION SHEET (to be read by, or explained to the patient)

Inflammatory bowel disease (IBD) causes intestinal symptoms and may also sometimes affect the joints.

Dr. Avinash is doing this study in order to find out what is the connection between the intestinal symptoms and the joint symptoms, and to see if the germs in the intestine or the patient's genes are responsible for these symptoms.

The study involves the normal clinical examination including a detailed examination of the joints. It also involves giving a 10 ml sample of blood for genetic testing and giving a stool sample for studying the germs in it.

All other tests are only done for your clinical care.

The results of the tests done in connection with the study may directly benefit you. They are likely to indirectly benefit other patients with the disease.

The results of the tests will be kept confidential and there will be no direct link of the test report with your hospital records.

You are free to not participate in this study if you do not so wish.

CONSENT

I hereby provide my consent for the above research study and to give the necessary information and samples.

I also provide my consent for the blood and stool sample to be preserved for additional research for a period of five years without my further approval, under the condition that the results will be kept anonymous and not linked to me.

Name:

Hosp. No:

Study No:

Date:

Signature:

Witnessed by:

(Name

)

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ABSTRACT

TITLE OF THE ABSTRACT: Joint manifestations in Inflammatory Bowel Disease (IBD) – A cross-sectional study with special emphasis on relationship of gut flora to bowel and joint disease

DEPARTMENT: Gastrointestinal Sciences

NAME OF THE CANDIDATE: Dr Avinash B

DEGREE AND SUBJECT: Doctor of Medicine (D.M) Medical Gastroenterology

NAME OF THE GUIDE: Dr B S Ramakrishna. DM, Professor and Head, Clinical Gastroenterology and Hepatology, Department of Gastrointestinal Sciences, Christian Medical College, Vellore

OBJECTIVES : To assess the frequency and pattern of joint involvement in inflammatory bowel disease and to determine whether joint involvement is associated with a dysbiosis of the intestinal microbial flora

METHODS: Consecutive patients with ulcerative colitis (UC) or Crohn's disease (CD) were prospectively interviewed and examined by the principal investigator and clinical details and laboratory results noted. The pattern of joint involvement and severity was independently confirmed by a specialist rheumatologist. Radiographs of the involved peripheral joints, sacroiliac joints and spine were obtained. DNA was extracted from stool samples and real time PCR targeting 16S ribosomal DNA was used to quantitate specific classes of “beneficial” (*Clostridium coccoides*, bifidobacteria, and lactobacilli) and “aggressive” (*Bacteroides*, Enterobacteriaceae, Enterococcus) bacterial flora. Disease

activity and bacterial counts were compared between patients with and without joint involvement.

RESULTS : Sixty eight patients with ulcerative colitis (UC) and 60 with Crohn's disease (CD) were included in this prospective study. Arthritis was noted in twenty- four IBD patients (18 %); 13 with CD (21.6%) and in 11 with UC (16.1%). Peripheral arthritis alone was noted in 12 patients, axial involvement alone in 7 and rest had mixed pattern. The most prevalent phyla in IBD patients were Bacteroidetes and Bifdobacterium.. Enterobacteriaceae were significantly higher in patients with UC than in CD. In UC patients, joint involvement was associated with higher Enterococcus species and lower Clostridium coccoides suggesting a dysbiosis in these patients.